	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	2043	thyroglobulin$1	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/03/18 16:11			0
2	BRS	L2	105273	lectin$1 or antibod$3	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/03/18 16:12			0
3	BRS	L3	1637123	quantif$7 or measure$4	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/03/18 16:13			0
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L1	2473182	S	LECTIN# OR ANTIBOD###
L2	29504	S	THYROGLOBULIN
L3	29975	S	THYROGLOBULIN#
L4	4627077	S	QUANTIFICAT### OR MEASUR####
L5	662	S	L1 (30A) L3 (30A) L4
L6	237		DUP REM L5 (425 DUPLICATES REMOVED)
L7	5634680	S	CANCER OR TUMOR OR TUMOUR OR MALIGNAN#### OR NEOPLAS###
L8	19	S	L7 (30A) L6
L9	106194	S	QUANTIFY
L10	9	S	L1 (30A) L3 (30A) L9

STIC-ILL 337,299

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ACTA ENDOCRINOLOGICA, (1985) Vol. 108, pp. 151

Eur J Nucl Med, (1981). Vol. 6, No. 11, pp. 515-520

ENDOCRINOL SUPPL. (1985) 108 (267), 151

J SAITAMA MED SCH, (1989) 16 (3), 353-364

CANCER RESEARCH, (1975 Oct) 35 (10) 2689-92

KLINISCHE WOCHENSCHRIFT, (1982 May 3) 60 (9) 457-64

CHINESE MEDICAL JOURNAL, (1989 Apr) 102 (4) 282-9

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION, (1990 Oct) 13 (9) 737-42

Thanks,

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埼玉医科大学雑誌 第16巻 第3号 平成元年7月

原 著

抗ヒトサイログロブリンモノクローナル抗体の作成とその応用

三 浦 志 朗

Production of Anti-Human Thyroglobulin Monoclonal Antibodies and Their Applications

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Thyroglobulin (Tg) is the main protein in the thyroid follicle, and the serum levels of Tg are increased in various thyroid diseases. However, in the common immunoassay system using polyclonal anti-thyroglobulin antibody (P-ATA), it has been difficult to measure serum Tg levels accurately in the patients who possess anti-Tg autoantibody (auto-ATA). Also, it has been impossible to differentiate Tg from neoplastic tissue and Tg from normal tissue. To solve these problems, I established twelve monoclonal anti-Tg antibodies (M-ATA).

All M-ATA belonged to IgG1k. These M-ATA were classified into six groups according to the cross-reactivity with auto-ATA. We developed a sandwich-type enzyme linked immunosorbent assay (ELISA) system for Tg using M-ATA as a first antibody. This ELISA showed very low background and high sensitivity (0.1 ng/ml).

Using this ELISA, I measured serum Tg levels in 18 cases with autoimmune thyroid diseases who had auto-ATA in their sera. The serum Tg levels were within the normal range in all cases. I also measured serum Tg levels in cases with thyroid cancer (n=15) and thyroid adenoma (n=15), using P-ATA and two M-ATA (D6 and E1). Different ATA resulted in different Tg levels, which suggested that there were some immunologic heterogeneity in serum Tg in patients with thyroid neoplasms. However, I could not detect any tumor-specific Tg.

To investigate the reactivity of M-ATA to tissue Tg, I also conducted immunohistochemical staining for Tg in cases with thyroid cancer (n=9) and thyroid adenoma (n=9) using M-ATA (D6) and P-ATA. Non-specific background staining was negligible when M-ATA was used. In a case of follicular cancer, serum Tg level was low despite the tissue Tg being heavily stained. In another case of papillary cancer, there was a discrepancy between the staining with P-ATA and that with M-ATA (D6).

I tried to detect anti-idiotypic antibody against idiotypes of five kinds of M-ATA (D1, D3, D6, E1 and G9). We could not detect any anti-idiotypic activity in 30 cases with autoimmune thyroid diseases in various disease stages.

I concluded that (1) the Tg levels in the patients with autoimmune thyroid siseases who had auto-ATA were low, (2) there were immunologic heterogeneities in serum Tg and tissue Tg in patients with thyroid neoplasms, (3) it seemed unlikely that anti-idiotype antibodies played major in the pathophysiology of autoimmune thyroid diseases.

Key words: thyroglobulin, monoclonal antibody, ELISA
J. Saitama Med. School, 16, 353-364, 1989
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埼玉医科大学第四内科学教室
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緒 言

モノクローナル抗体は，抗体の特異性，親和力，

クラス，サブクラス，などその均一性から，不均一な抗体を含む免疫血清，すなわちポリクローナル抗体と比べ，より詳細な抗原の解析を可能とし，広く応用されつつある．

甲状腺濾胞の主要蛋白であるサイログロブリン（Tg）は，分子量66万の巨大な糖蛋白で，各種甲状腺疾患患者及び正常人血中にも見いだされ，その測定は，各種甲状腺疾患の病態解明に多くの有用な情報を与えてきた[1-3]．血中 Tg の測定には，我々の教室で開発された高感度エンザイムイムノアッエイ（EIA）[4,5]をはじめ，ラジオイムノアッセイ（RIA）[6]，及び EIA[7] が各施設で利用されている．

現在，Tg 測定の臨床上の問題点としては，まず，抗 Tg 自己抗体（Auto-ATA）陽性患者における血中 Tg 測定の困難性があげられる．つまり測定に用いる免疫家兎ポリクローナル抗 Tg 抗体（P-ATA）と，Tg との結合を，血中の Auto-ATA が，競合阻害してしまう為，Auto-ATA 共存下での Tg の直接の測定は困難とされる．P-ATA を用いる場合，Tg の% recovery を計算し，その理論値を求める事は可能であるが[5,8]，非常に煩雑である．そこで，Auto-ATA と抗原認識部位の異なるモノクローナル抗ヒト Tg 抗体（M-ATA）を用いれば直接測定が可能となると考えられる[9,10]．第2に甲状腺腫瘍における血中 Tg 測定に関する諸問題がある．甲状腺癌患者において血中 Tg の経時的測定は，術後再発の発見目的で高い有用性が報告されている[11,12]．しかし，遠隔転移のない甲状腺乳頭腺癌では，その約30%前後で，通常の測定では血中 Tg 値の上昇をみない．このような症例では，血中での Tg は特殊な形に変形し，通常の抗体とは反応しない状態になっている可能性がある．このような特殊な Tg と反応する M-ATA が存在すれば，その臨床的価値は高いものと考えられる．さらに最近，Sikorska[13] は，自己免疫性甲状腺疾患患者血中に M-ATA に対する抗イディオタイプ抗体を見いだした．免疫調節の観点から，このような抗イディオタイプの病因ならびに病態との関連を追求することは意義のあることと考えられる．

以上をふまえ，筆者は M-ATA を作成し，次の点について検討を行った．

(1)各 M-ATA の特性．(2) EIA（サンドウィッチ法）による血清 Tg の測定．特に Auto-ATA 陽性患者血清 Tg 測定及び甲状腺腫瘍特異的 Tg の検索．更に血清 Tg 値と組織 Tg の染色性の相違の検討を試みた．(3)加えて，自己免疫性甲状腺疾患患者における M-ATA に対する抗イディオタイプ抗体の検索も行った．

対象と方法

（対象）

血清及び組織切片はすべて埼玉医科大学第四内科甲状腺外来及び入院の患者より採取した．Auto-ATA 陽性患者は，Graves 病（GD）及び橋本病（HD）の患者から選んだ．GD と HD の診断は，通常の臨床症状，甲状腺機能，THS レセプター抗体，抗 Tg 抗体，抗マイクロゾーム抗体，等により行った．競合阻害試験に用いた Auto-ATA の IgG 分画は，GD 4例，HD 6例，計10例から，後述のように作製した．抗イディオタイプ抗体の検索に用いた IgG 分画は，上記の IgG 分画も含めて GD 15例，HD 15例，計30例から，後述の方法で作製した．甲状腺癌患者については，血中 ATA 陰性で，手術後病理診断で確認された者とした．甲状腺良性腫瘍腫については，触診，画像診断，穿刺吸引細胞診より良性と診断した患者血清，ならびに手術により確認された患者の血清を使用した．

（方法）

1. 抗原（ヒト Tg）の精製

ヒト Tg は，Graves 病患者甲状腺組織ホモジネートから，Derrien らの硫安塩析法[14]で分離し，Sephacryl-S 300のカラムで精製した．蛋白濃度は Bio Rad 法及び分子吸光度係数を用いて測定した．

2. M-ATA の作成[15]

6週齢雌の BALB/C マウス腹腔内にヒト Tg 100μg/100μl リン酸緩衝液（PBS）/マウスと等量の complete Freund's adjuvant を混合した emulsion を1週間毎に2回注射した．2回目の注射の3日後，眼静脈より採血し，血中抗体価を ELISA 法（後述）にて確認した．高抗体価のマウス

には，10日後に，ヒト Tg 50μg/100μl/マウスで追加免疫をし，その3日後，細胞融合を行った．低抗体価のマウスは，更に同量で免疫を繰り返し，高抗体価となったところで追加免疫をし，細胞融合に使用した．

細胞融合は，免疫されたマウス脾細胞とマウス骨髄腫細胞(P 8-X63-AG865)を5：1の比率で50% Polyethylene glycol 4000 (MERCK 社製)の存在下で，2分間 Vortex で攪はんして行った．

融合した細胞は，Hybridoma 選択培地である Hypoxanthine-Aminopterine-Thymidine (HAT) 添加15%胎児牛血清 (FCS) RPMI1640に，脾細胞として 5×10^6個/ml の割合で再浮遊し，96穴 micro plate (Immunoplate II，NUNC 社) で培養した．10日め頃より HT (Hypoxanthine-Thymidine) 添加培地に移行し，融合より約2週間後，コロニーが観察されたウェルの上清を ELISA (後述) 法にて抗体スクリーニングを行った．抗体陽性かつ増殖良好なウェルを選び，限界希釈法にてクローニングを行った．Feeder 細胞として4-5週齢—BALB/C マウスの胸腺細胞を 2×10^6/well で使用した．更に2～3週間培養し，1ウェルに1個のコロニーが増殖したウェルを選び再び上清の抗体のスクリーニングを行った．抗体スクリーニングとクローニングの操作を3回繰り返したうえで M-ATA 産生 Hybridoma とした．

3. M-ATA の精製

作成した12種の M-ATA 産生 Hybridoma は，ボトル内あるいは BALB/C マウス腹腔内で大量培養した．培養上清，あるいは腹水を硫安で濃縮し，透析後，Protein A CL4B カラムにて IgG を精製した[16]．更に Mouse typer kit (Bio-rad 社製) を用いて各々のクラス，サブクラスを確定した．M-ATA は，凍結乾燥，あるいは5%ウシ血清アルブミン (BSA) 添加- PBS 0.1% Tween20 (希釈緩衝液) で1 mg/ml とし，−20°Cで保存した

4. P-ATA の作製

精製 Tg (5 mg/ml) と等量の complete Freunds's adjuvant を家兎足底及び背筋に注射し，2週毎に3回追加免疫し，抗 Tg 血清を得た．この血清から DEAE Sephadex A-50カラムで IgG 分画を採取し，その後 CNBr 活性化 Sepharose 4B (Pharmacia 社製) に Tg を結合させた affinty カラムにて純化精製した．

5. 患者血清中 IgG の精製

DEAE-Sephadex A50カラムを用いて，患者血清より IgG を分離精製した．競合阻害試験に使用した10検体は，抗体価が抗体凝集反応 (Serodia-ATG，Fujirebio) を用いて1,600倍から6,400倍のものを使用した．

6. Biotin 標識 ATA の作製[17]

0.1M $NaHCO_3$ 1 ml に1 mg/ml の P-ATA あるいは M-ATA を溶解し，Dimethyl sulfoxide (DMSO) に溶解した Biotinyl-N-Hydroxysaccinimide (BNHS： 1 mg/ml) 60μl を混合する．室温で4時間インキュベート後，4 °C 一晩 PBS で透析し，Biotin 化 ATA とした．

7. ペルオキシダーゼ (PO) 活性の測定

PO は，0.1Mクエン酸，0.2Mリン酸水素2ナトリウム緩衝液，pH 4.8，50ml と o-phenylendiamine 20mg，30% H_2O_2 10μl 混和し，基質液とした．各ウェルに200μl ずつ基質液を加え，室温で30分インキュベート後，6 N硫酸 50 μl を入れ，反応を停止させ，OD 492で PO 活性を測定した．

8. ATA のスクリーニング法

精製した Tg を0.1M炭酸—重炭酸緩衝液，pH 9.4 (C-B 緩衝液) で，100μg/ml に希釈し，96穴 microplate (固相) に50μl ずつコートし，室温で60分間インキュベートした．0.1% Tween 20加 PBS (洗浄緩衝液) で3回洗浄後，Hybridoma 培養上清または，免疫したマウス血清の100倍希釈液100μl を加え，37°C，2時間インキュベートした．洗浄後，希釈緩衝液で2,000倍希釈した Biotin 標識抗マウス IgG (Vector 社製) を100μl 加え，37°C 1時間反応させ，洗浄した．その後，希釈緩衝液で50万倍希釈した PO 標識 Avidin (Vector 社製) を100μl 加え，37°C 1時間反応させ，洗浄後，PO 活性を測定した．background には，培養上清のかわりに15% FCS RPMI1640 (培養液) をおき，OD 492が background の8倍以上の場合を抗体陽性ウェルとした．

ラット Tg との交差反応は，ヒト Tg のかわりに，精製した正常ウイスターラット Tg 100μg/ml

を50μl 固相にコートし，次に M-ATA を含む培養上清を加え，同様の操作で測定し，ヒト Tg をコートした場合と比較し，検討した．

9. 競合阻害試験

精製したヒト Tg を0.1M C-B 緩衝液で100μg/ml に希釈し，96穴 microplate に50μl ずつコートし，室温で60分間インキュベートした．洗浄緩衝液で3回洗浄後，各々の Auto-ATA 陽性 IgG を原液（370μg/ml）から希釈緩衝液にて段階希釈し，各100μl ずつ加え，37°C で90分間反応させた．洗浄後，各 Biotin 標識 M-ATA を予備実験にて決定した至適濃度で希釈して100μl ずつ加え，37°C で90分間反応させて洗浄した．次に20万倍希釈 PO 標識 Avidin を加え，37°C 90分間反応させ，洗浄後，PO 活性を測定した．原液の Auto-ATA 陽性 IgG を competitor として加えた OD 492が，希釈緩衝液を competitor として加えた OD 492の何%であるかを計算し，その競合抑制率により，Auto-ATA と各 M-ATA の交差性を決定した．

10. 血中 Tg の測定及び標準曲線の作製

谷川の方法[5]を一部改変したサンドウィッチ ELISA 法を用いた．すなわち M-ATA のうち Auto-ATA との交差性の異なる3種（D6, E1, D1）を C-B 緩衝液で50μg/ml に希釈し，96穴 microplate に50μl ずつコートした．4°C 一晩インキュベーション後，残った M-ATA を除去した．洗浄後，blocking 操作として，希釈緩衝液を各ウエルに100μl ずつ加え，30分室温でインキュベートした．吸引後，FCS で希釈した Tg 標準液あるいは血清を原液のまま50μl を加え，37°C，2時間インキュベートした．洗浄後，希釈緩衝液で至適濃度に希釈した Biotin 化 P-ATA を150μl 加え，37°C で1時間反応させた．更に洗浄後，30万倍希釈した PO 標識 Avidin を150μl 加え，1時間反応させた．洗浄後，PO 活性を測定した．

各検体は，二重測定で測定し，Biotin 標識 P-ATA の至適濃度は，予備実験により決定した．

11. 抗イディオタイプ抗体の測定

作製した12個の M-ATA のうち，大量培養可能であった5種（D1, D3, D6, E1, G9,）のイディオタイプに対する抗イディオタイプ抗体の測定を試みた．自己免疫性甲状腺疾患患者30人の血清より前述の方法にて IgG を精製した．すなわち，(1) Auto-ATA 1,600倍から6,400倍まで安定している10例（GD 5例，HD 5例），(2) Auto-ATA 25,600倍以上（GD 1例，HD 3例）．(3)疾患経過中に Auto-ATA の値が減少（GD 1例，HD 3例）．④Auto-ATA 陽性であるが，従来のP-ATA を用いて Tg 測定可能（GD 1例）(5)Auto-ATA 陰性であるが，従来の P-ATA を用いて Tg 測定感度以下（GD 2例，HD 3例）．(6) Auto-ATA 陰性で Tg が 20ng/ml 以上（GD 6例）．これらの IgG は，希釈緩衝液で 5μg/ml に希釈した．測定手順は，96穴 microplate に，C-B 緩衝液にて 50μg/ml に希釈した M-ATA 50μl をコートし，4°C，一晩インキュベートした．洗浄後，希釈緩衝液で blocking 操作を行い，緩衝液を吸引した．ここに精製した上記の IgG を加え，37°C 2時間インキュベート洗浄緩衝液で洗浄した．その後，マウス血清を 0.5% 添加し，適当に希釈した PO 標識モノクローナル抗ヒト IgG 抗体（ZYMED 社製）（第二抗体）を加え，1時間インキュベートした．洗浄後，PO 活性を測定した．

第二抗体は，陽性対照として，ヒト Tg 50μg/ml を固相にコートし，同様の方法で測定した場合の OD 492が，1,000以上となるように希釈した．

陰性対照は，(1) 固相に希釈緩衝液のみをコートし，他は同様の操作で行った場合，(2) 固相にヒト Tg 50μg/ml，次に M-ATA 1.0μg/ml，次に第二抗体とした場合，(3) 固相は M-ATA で，患者 IgG の代わりに正常人より精製した IgG を用いて測定した場合，とした．

12. M-ATA による組織染色

手術にて摘出した甲状腺腫瘍をパラフィン固定し，その薄切切片を ABC キット（VECTASTAIN 社製）にて酵素（PO）免疫染色した．

結　果

1. M-ATA の特性

細胞融合は，5回行い，第4回目の細胞融合で，8種のクローンができ，精製された（D1, C8, B7, A9, G7, G10, B11, G9）．次に，第5回目の細胞融合で 4種のクローンが精製された（A8,

Table 1 Cross-reaction of monoclonal ATA (M-ATA) with patients' ATA (Auto-ATA)

Pt	Auto-ATA (TGHA) titer	D1	A9	A8	D6	C8	D3	B7	B11	E1	G9	G10
Y. S.	×6400	+	+	+	+	+	+	+	+	+	+	+
T. T.	×1600	+	+	+	+	+	+	+	+	+	+	+
N. S.	×6400	+	+	+	+	+	±	+	+	+	+	+
S. T.	×6400	+	+	+	+	±	±	+	+	+	+	−
K. Y.	×6400	+	+	±	±	±	±	±	±	±	±	−
N. M.	×1600	+	±	±	±	±	+	−	−	−	−	−
S. S.	×1600	+	±	−	−	−	−	−	−	−	−	−
O. A.	×6400	±	−	−	−	−	−	−	−	−	−	−
K. K.	×1600	−	−	−	−	−	−	−	−	−	−	−
Y. Y.	×1600	−	−	−	−	−	−	−	−	−	−	−

$$\% \text{ Inhibition} = \left(1 - \frac{\text{OD492nm in the co-presence of Auto-ATA IgG}}{\text{OD492nm in the co-presence of 5\% BSA PBS}}\right) \times 100$$

+ (% inhibition 50%) denotes the presence of a cross-reaction.
± (20% < % inhibition < 50%) denotes the presence of a questionable cross-reaction.
− (% inhibition 20%) denotes the absence of a cross reaction.

D3, D6, E1). 大量培養中, M-ATA G7は失活し, 計11種となった. これらは全て IgG 1Kに属した. データは示さないが, どの M-ATA も, 正常ラット Tg とは反応しなかった. これに対し, P-ATA の場合は, ラット Tg をコートした場合もヒト Tg をコートした場合と同程度の結合性が認められ, ラット Tg とも交差することが確かめられた.

2. Auto-ATA 陽性 IgG との交差反応性 (Table 1).

11種の M-ATA は, 無作為に選んだ, Auto-ATA が1,600倍から6,400倍の患者 IgG との交差反応性の違いで6群に分けられた. すなわち, competitor として同量の希釈緩衝液を用いた場合の OD 値を100%とし, 各々の Auto-ATA 陽性 IgG を competitor とした場合の OD 値が, 50%以上抑制されるものを (+), 20%から50%のものを (±), 20%以下のものは, ほぼ抑制がないものと考え, (−) とした. すなわち, (+) のものは交差反応あり, (±) は不十分, (−) はなしと考えられる. 今回の11種の M-ATA は, 少なくともどれかの Auto-ATA と交差反応を示し, 10人全ての Auto-ATA と交差しないものは認められなかった.

3. ヒト血清 Tg 測定系に関する検討

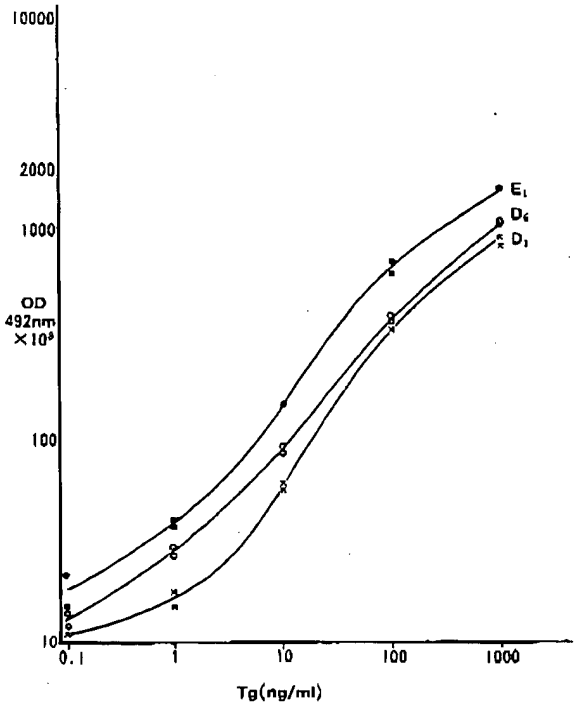

Fig. 1 Standard curve of Tg-ELISA using M-ATA (D1, D6 and E1).

Fig. 1 に, 今回の M-ATA を用いた Tg 測定の標準曲線を示す. 固相の M-ATA は, Table. 1 に示すように群が異なり, しかも大量培養可能

Table 2 Precision of Tg-ELISA using M-ATA (D6)

	Sample 1	Sample 2	Sample 3	Sample 4
Intra-assay				
n	12	12	12	12
Mean±SD	271.7±11.7	165.8±10.4	25.6±2.1	7.2±0.5
CV(%)	4.3	6.3	8.2	6.9
	Sample 5	Sample 6	Sample 7	Sample 8
Inter-assay				
n	6	6	6	6
Mean±SD	254.5±26.2	111.7±8.8	14.3±1.9	9.8±12.3
CV(%)	10.3	7.9	13.0	12.3

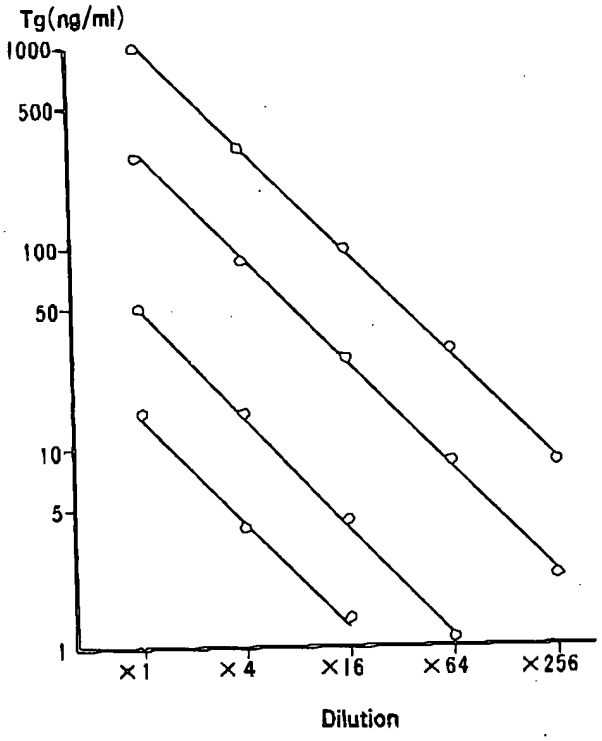

Fig. 2 Dilution study in Tg-ELISA using M-ATA (D6).

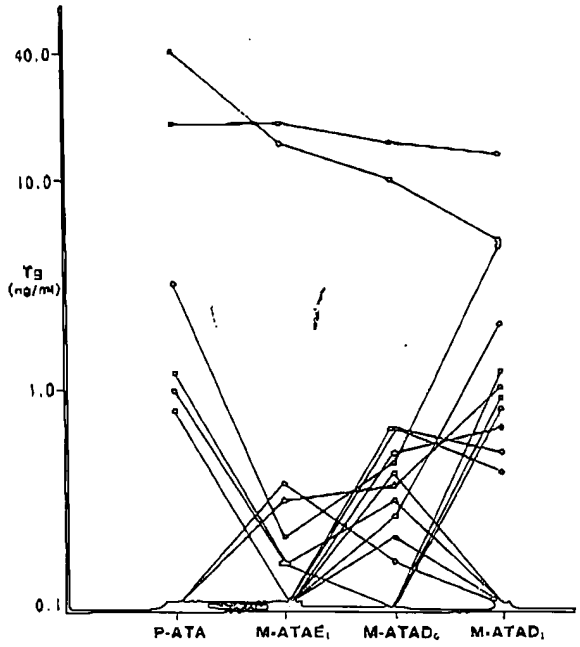

Fig. 3 Measurement of thyroglobulin in auto-ATA positive sera using P-ATA and the different type of M-ATA (E1, D6 and D1).

であった3種 (D1, D6, E1) を用いた. Tg 0 ng /ml を比較すると, M-ATA (D1, D6, E1) それぞれで, 常に0.1ng/ml の方が高値を示し, 0.1ng /ml までは測定可能と考えられた. また, M-ATA と P-ATA で比較すると, background の OD 値は, M-ATA の方が明らかに低値であり, background と, 0.1ng/ml との比率も M-ATA の方が大きく, M-ATA を使用した測定系の方が敏感であると考えられた. 各種血清における本測定の変動係数は, M-ATA (D6) を用いた測定系で検討した結果, Table. 2 に示すように, 重複変動 (intra-assay) で4.3%～7.9%, 日差変動 (inter-assay) で7.9%～13.0%を示した. FCS を用いて4倍から256倍まで希釈した後, 測定した結果 (Fig. 2) は, 良好な平行性と直線性を示した.

4. Auto-ATA 陽性血清中 Tg の測定

Fig. 3 に3種の M-ATA 及び従来の P-ATA を用いて測定した Auto-ATA 陽性血清中 Tg を示す. 結果は, 測定可能であった Tg 値のほとんどが過去に報告された正常値 (Van Herle[6]: <

20.7ng/ml, Endo[7]: <59.5ng/ml, Hara[4]: <50.0ng/ml, Tanikawa[5]: <47.4) を下回っていた. また, Tg 値が測定可能な例は, P-ATA で 6/18, M-ATA で E1: 7/18, D1: 11/18, D6: 12/18, と, M-ATA を用いた測定で増加する傾向がみられた. しかし, 同じ血清でも, D1のみで測定可能な Tg, あるいはD6とE1で測定可能で, 他では測定不能な Tg 等, 多様性がみられた. P-ATA のみで Tg 測定可能な血清は1例もなく, P-ATA で測定可能なものは3種いずれかの M-ATA で測定できた.

5. 甲状腺腫瘍特異的 Tg の検討

Fig. 4は, P-ATA 及び, 2種の M-ATA (D6, E1) を用いて測定した, 甲状腺腫瘍患者の血中 Tg を示す. 甲状腺癌の血中 Tg の測定値は P-ATA と M-ATA (E1), P-ATA と M-ATA (D6) を用いた場合, 各々強い相関を認めた. ($r=0.98$, $r=0.93$). 良性腫瘍の血中 Tg も同様に P-ATA と M-ATA (E1), P-ATA と M-ATA (D6) の測定で相関を認めた ($r=0.98$, $r=0.88$). しかし, 個々の例をみると, P-ATA より M-ATA での測定の方が高値を示す例, 逆に P-ATA で高値を示す例等が認められた. しかし, 癌 (乳頭腺癌9例, 濾胞腺癌6例) あるいは良性腺腫中で, ある抗体に特異的に反応を示す Tg の存在は認められなかった. また, Fig. では示さないが P-ATA, M-ATA のいずれで測定しても Tg 0.1ng/ml 以下の例もみられた.

6. 甲状腺腫瘍 Tg の組織学的検討

手術標本より得た甲状腺腫瘍 (良性腺腫3例, 濾胞腺腫4例, 乳頭腺癌5例) のパラフィン固定切片を P-ATA, M-ATA (D6) にて染色性の違いを検討した. Fig. 5の右側は全て M-ATA (D6), 左側は全て P-ATA を使用した染色である. 上段及び中段は, ある甲状腺濾胞腺癌患者の組織であるが, 上段は正常部分, 中段は腫瘍部分の染色で, 両部分とも, M-ATA でも P-ATA でも良好な染色性を示した. しかし, 血中の Tg 値は, P-ATA で0.5ng/ml, M-ATA (D6) で2.0ng/ml と非常に低値であり, 血中 Tg 値と組織染色性に矛盾が認められた.

最下段は, 他の甲状腺乳頭腺癌症例の腫瘍部分を示した. 左側の P-ATA を使用したものは一部染色されているが, 右側の, M-ATA で染色したものでは, 全く染まっておらず, M-ATA と P-ATA の染色性に相違が認められる. この例での血中 Tg 値は, P-ATA, M-ATA のいずれで測定しても20ng/ml と測定可能であった.

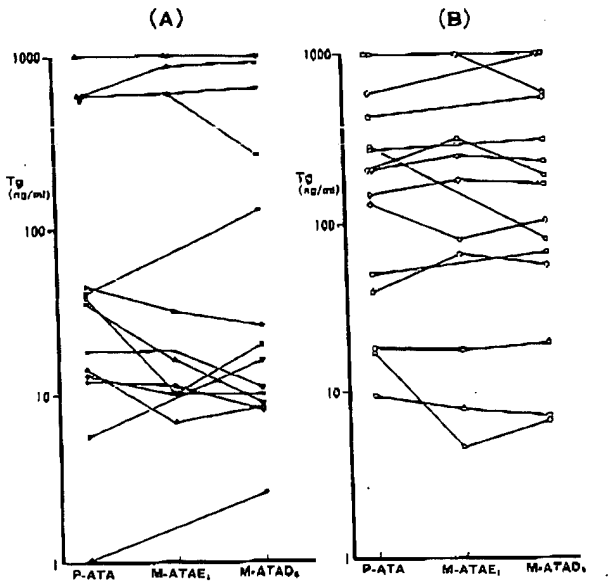

Fig. 4 Measurement of thyroglobulin in patients with thyroid cancer (A) and in those with thyroid benign tumor (B) using P-ATA and the two different types of M-ATA (E1 and D6).

7. M-ATA に対する抗イディオタイプ抗体の検索

今回検索を試みた30例の自己免疫性甲状腺疾患の患者 IgG には, 5種の M-ATA (D1, D3, D6, E1, G9) に対する抗イディオタイプ抗体は1例も認められなかった. この測定系は, 必ずしも抗イディオタイプ抗体のみでなく, 抗マウス IgG 抗体とも反応してしまうが, 以上のように全例陰性であった事から, これ以上の検討は行わなかった.

考 察

Tg は, 甲状腺細胞内で作られ, 濾胞内に貯蔵される分子量66万の巨大糖蛋白であり, ホルモン合成の場としての役割を持ち, 約40個の抗原決定基を持つといわれる. また正常人及び, 各種甲状腺疾患患者の血中にも見いだされるため, 甲状腺

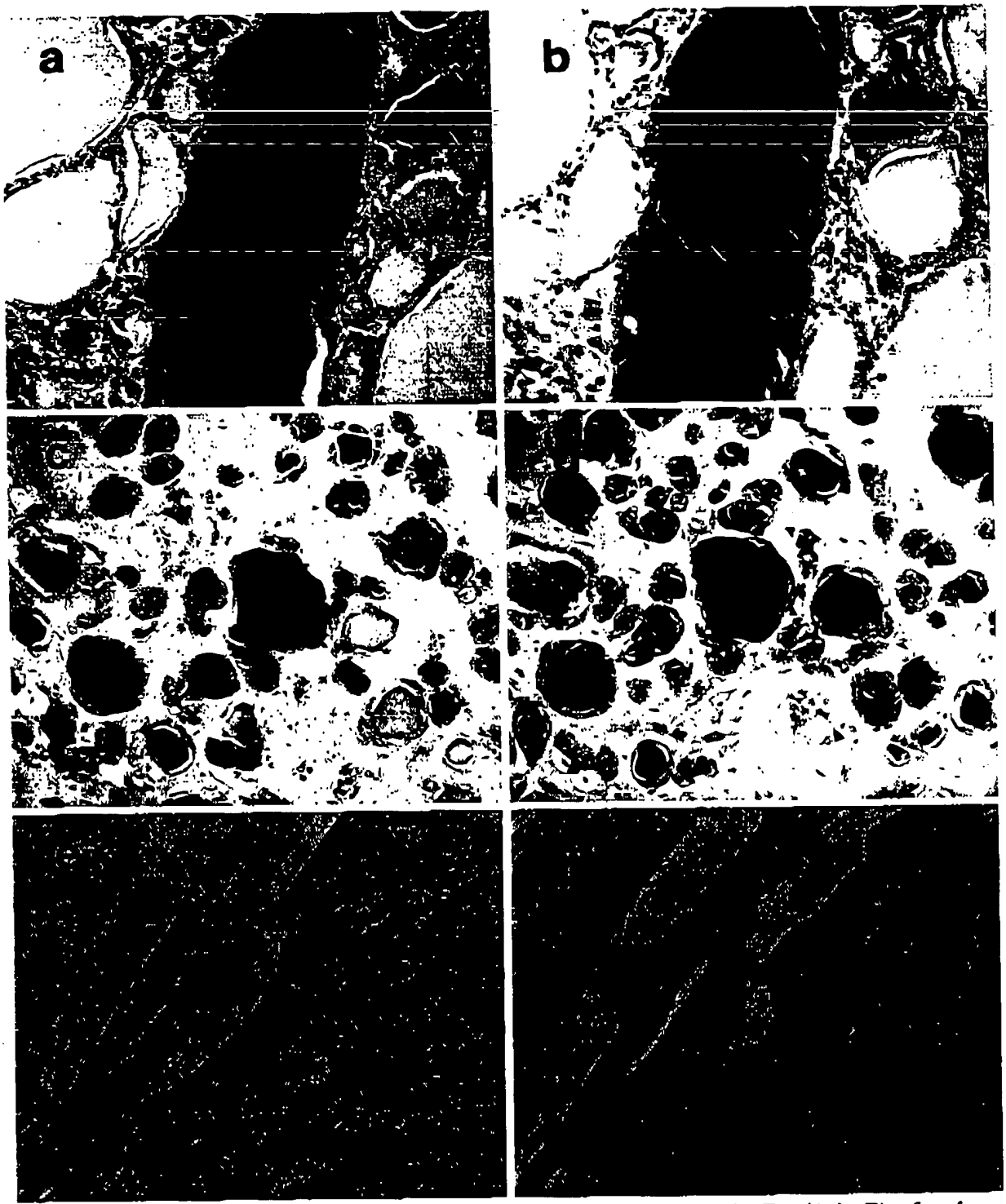

Fig. 5 Enzyme-immunostaining for thyroglobbulin using P-ATA and a M-ATA (D6). The first four sections (a, b, c and d) were obtained from a patient with follicular cancer, who hada very low serum Tg concentration in spite of Tg being heavily stained with both P-ATA and M-ATA. a. A section of normal thyroid tissue stained with P-ATA (200×) b. A section of normal thyroid tissue stained with M-ATA (200×) c. A section of normal thyroid tissue stained with P-ATA (100×) d. A section of follicular cancer tissue stained with M-ATA (100×) e. A section of papillary cancer tissue stained with P-ATA (100×) f. section of papillary cancer tissue stained with M-ATA (100×)

疾患の病態解明に様々な面から利用されている．今回作製した11種の M-ATA は全て，ヒト Tg と強い親和性を有し，ラット Tg とは交差せず，IgG1k に属していた．これらの M-ATA は，Auto-ATA との交差性によって6群に分類された．今回の11種の M-ATA に関しては，10人の患者全ての Auto-ATA と交差しないものは認められなかった．しかし，群の異なる M-ATA を用いて Auto-ATA 陽性患者血中 Tg を測定し，従来の P-ATA での測定値との違いを検討することで臨床応用を試みた．

まず，Tg 測定系についてであるが，谷川の報告した，Avidin-Biotin System を利用したサンドウィッチ ELISA 法[5]を用いた．本法は，Avidin が，強い親和性をもって Biotin と特異的に結合することを利用した高感度 Assay である．今回この方法の，固相の抗体に P-ATA の代わりに，M-ATA をコートし，抗体濃度，反応時間，サンプル量等に改良を加えて測定した．測定感度は 0.1ng/ml で，過去に報告された M-ATA を用いた血中 Tg 測定感度，すなわち，Kato ら[9]の2 ng/ml, Strongkul ら[10]の1.0ng/ml, Narkar ら[18]の7.8ng/ml より良好で，P-ATA を用いた谷川と同等であった．また，Tg 0ng/ml の OD 値，すなわち background は，OD492が0.010以下で，P-ATA を用いた場合の約1/7であり，非特異的反応を十分に減少させることができた．この事より標準曲線は，Tg 1.0ng/ml 以下の部分でも直線に近く，Table には示さなかったが，Tg 1.0ng/ml 以下の検体でも M-ATA (D6) を用いた測定で，重複変動 (intra-assay) で平均0.98±0.22ng/ml，変動係数22.4%と通常の測定に使用可能な範囲で測定できた．今後，更に改良を加えれば，0.1ng/ml 以下の Tg 値も測定可能となるかもしれない．また，本法は RIA と比べ安価で，検体量は，二重測定で測定しても100μl と少量ですみ，用いる M-ATA, Biotin 標識 ATA, HR-PO 標識 Avidin も −20°C で長期保存が可能な事等の利点を有していた．

さて，一般に Auto-ATA 陰性の GD や HD の症例では，血中 Tg 値の上昇をみるが[5]，この測定系を利用し，無作為に選んだ Auto-ATA 陽性患者血中 Tg 値を測定したところ，Tg 測定可能な例数は，M-ATA で増加する傾向があったが，値はいずれも正常範囲であった (Fig. 3)．この理由としては，まず当然 M-ATA が Auto-ATA と Tg 認識部位を共有しており，Auto-ATA により，測定が阻害されたことが考えられる．次には，分泌された Tg が，Auto-ATA と immunecomplex (TgIC) をつくり[19,20]，急速に血中から消失してしまう可能性が考えられる．従来の Auto-ATA 存在下での Tg 測定の報告では，Bayer ら[6]は，外から過剰の Tg を加えて Tg を測定し，Auto-ATA の影響を除外した% recovery を求め，Tg の理論値を算出し，Tg の実測値が 10ng/ml 以下の例では，全例理論値も正常範囲内であったと述べた．また，Srintongkul ら[10]は，Auto-ATA に影響されない M-ATA を用いて Tg を測定し，Auto-ATA 陽性 GD 患者では，陰性患者と比べ，Tg が低値であり，外から投与した Tg の半減期が短縮していると報告した．これは，これらの患者では TgIC が形成され，血中からより速く消失することを示唆していると考えられる[5,21]．

また，P-ATA, M-ATA で，血中 Tg 値に差を認めた．これは第一に，各 M-ATA による抗原認識部位が異なり，Auto-ATA も個々の例が全て，Tg の同じ部位を認識していない可能性がある事が考えられる．第二に，各 M-ATA, P-ATA の Tg に対する親和性の違いにより測定値に差がでているとも考えられた．

甲状腺腫瘍特異的 Tg の検索については，Fig. 5にみられるように，今回2種の M-ATA を用いた測定では，癌，あるいは良性腺腫に特異的な Tg を認識している M-ATA は見いだせなかった．しかし，個々の例を見ると，甲状腺癌でも良性腺腫でも，今回の3種の測定間で，測定誤差とは考えられないような相違が認められた例も存在した．それらの症例では，血中 Tg の抗原性が，標準 Tg と異なっていたのではないかと想像させる．この点に関して，以前より腫瘍 Tg と正常 Tg の相違がいくつか報告されている．Monaco ら[22]はラット甲状腺腫瘍の Tg は，正常部と比べヨード含量が低いと報告している．その後，Sch-

neider ら[23]はヒトの甲状腺良性，悪性腫瘍患者での血中 Tg のヨード含量の低下を証明している．また Izumi ら[24]は，ラット甲状腺腫瘍部の Tg は炭水化物含量が低いと報告している．さらに，Heilig ら[25]，Hüfner ら[26]は，M-ATA と P-ATA の2法を利用して，甲状腺腫瘍患者の血中 Tg を測定し，2法の測定値がきれいには相関しないことから，彼らは甲状腺腫瘍 Tg の不均一性を示唆した．以上より，全ての甲状腺癌に共通してみられ，正常甲状腺にみられない甲状腺腫瘍特異的 Tg が存在するか否かはさだかではないが，現段階ではむしろ，P-ATA，M-ATA で測定した値の違いから良性腺腫，乳頭腺癌，濾胞腺癌を鑑別していく方法を検討していくべきと考える．

甲状腺腫瘍患者の血中 Tg の不均一性が示唆されたので，次に，組織の腫瘍部と正常部の Tg を P-ATA と M-ATA (D6) で染色し，抗原性の違いを検討した．今回の M-ATA を用いた染色は，組織染色性は良好で，非特異的染色は少なく，このような組織学的検討に適したものと考えられた．

まず，検索した12例中1例（濾胞腺癌症例，Fig 5, a, b, c, d）で組織染色性と血中 Tg 値の間に解離を認めた．すなわち，癌組織の染色性は極めて良好で，かつ血中には Auto-ATA が存在しないにもかかわらず，血中 Tg 値は低い場合もあることが裏づけられ，興味ある所見であった．

次には，P-ATA では一部染色されるが，M-ATA (D6) では，全く染色されない乳頭腺癌の例がみられた．もし，癌組織から Tg が血中へ分泌されているとすれば，P-ATA で高値で，M-ATA で正常値となることが予想される．しかし，本例の血中 Tg 値は20ng/ml で，P-ATA，M-ATA のどちらで測定しても同様であった．従って，本例の血中 Tg は正常部より分泌されたものだけが測定されていたと考えられた．また，本例の腫瘍 Tg は，M-ATA と反応する epitope を欠いていることが想像される．

Kurata ら[27]は6種の M-ATA を作製し，組織染色を行い，その染色性で2群，すなわち，甲状腺濾胞と上皮細胞の両方が染色される群，及び上皮細胞中心に染色される群に分類した．前者をヨード化関連 epitope を認識する M-ATA 群，後者をヨード化非関連 epitope を認識する M-ATA 群と考えた．この所見と Schneider ら[23]の，甲状腺腫瘍患者の血中 Tg 値が，ヨード含量が低いという報告と合わせ，その特異性を見いだしうるのではないかと言及した．今後，更に多くの M-ATA を用いて多くの染色組織を試みる事がこのような特異性を見いだすのに必要と思われる．

最近，Shepherd ら[28]は，M-ATA を I-123で標識し，甲状腺癌術後の患者に静注し，その集積像で甲状腺癌再発の診断に応用している．この方法は，I-123全身スキャンのように，甲状腺ホルモンの補充療法を中止する必要がなく，血中 Tg の測定と併用することで，今後の発展が期待される．我々の M-ATA もこのような臨床応用が今後可能と考えられる．

今回我々も，M-ATA の応用として，5種の M-ATA を用いて，様々な病期での自己免疫性甲状腺疾患患者 IgG について，抗イディオタイプ抗体の測定を試みた．しかし，1例も見いだすことはできなかった．

さて，Jerne ら[29]が提唱した Idio type network 説に基づき，甲状腺疾患においても最近，抗イディオタイプ抗体について検討されている．Zauali ら[30]は61人の多発性骨髄腫の患者で，その1名の血清中に Auto-ATA の $(Fab)_2$ に対する抗イディオタイプ抗体を見いだした．一方，甲状腺疾患について，Hara ら[31]は12人の GD，10人の HD 患者血清中に Auto-ATA の $(Fab)_2$ に対する抗イディオタイプ抗体は1例も見られなかったと報告している．Sikorska は[13]，GD 26名中2名，HD 40名中4名，RA 58名中7名の患者血清中に5種の M-ATA のうち1種の M-ATA に対する抗イディオタイプ抗体を，RIA，ELISA 法の両者で見いだしたが，正常人20名の血清中には見られなかったと報告している．更に，抗イディオタイプ抗体の投与によって，自己免疫性疾患の病因となりうる自己抗体のレベルを下げ，将来治療にも利用可能かもしれないと，言及している．

今回我々も，今後更に多数の M-ATA を用いて，実験していく必要があると思われた．

結　語

1) 12種の抗ヒトサイログロブリンクモノローナル抗体を作製した.

2) それらは全て IgG1k に属し，ラットサイログロブリンとの反応性は認められなかった.

また，Auto-ATA 陽性患者 IgG との交差性で6群に分類された.

3) これらを利用して，非特異的反応の少ない血中 Tg 測定の高感度 Sandwitch ELISA 法を開発した．それを利用して血中 Tg の測定を行った.

① Auto-ATA 陽性患者の血中 Tg 値は，3種の M-ATA を用いた測定では，いずれも低値だった．P-ATA を用いた場合より，血中 Tg 測定可能な症例数は増加する傾向がみられた.

② 2種の M-ATA を用いた測定では甲状腺腫瘍特異的 Tg は見いだせなかった．しかし，個々の症例で，使用する ATA により測定値に違いが認められた.

4) 12例の甲状腺腫瘍組織を P-ATA，M-ATA (D6) を用いて染色したところ，血中 Tg 値と組織染色性に矛盾のある例が1例，P-ATA と M-ATA (D6) の染色性に違いがあるものが1例認められた.

5) 5種の M-ATA に対する抗イディオタイプ抗体は，30名の自己免疫性甲状腺疾患患者には1例も認められなかった.

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文　献

1) Roit, I. M. & Torrigani, G.: Identification and estimation of undegraded thyroglobulin in human serum. Endocrinol., 81, 421-429, 1967.
2) Izumi, M. & Lasen, P. R.: Correlation of sequential changes in serum thyroglobulin, triiodothyronine, and thyroxine in patient with Graves' disease and subacute thyroiditis. Metabolism., 27, 449-460, 1978.
3) Uller, R. P. & Van Herle, A. J.: Effect of therapy on serum thyroglobulin levels in patients with Graves' disease. J. Clin. Endocrinol. Metab., 46, 747-755, 1978.
4) 原　義人，谷川俊則，柳沢守文，飯高　誠，坂詰良樹，根岸清彦，石井　淳，伊藤国彦：各種甲状腺疾患におけるヒト血中サイログロブリン値に関する研究．日内会誌，73，1451-1460，1984.
5) 谷川俊則：Avidin-Biotin system を用いた高高感度血中サイログロブリン測定法の開発とその臨床応用．日内分泌会誌，64，402-418，1988.
6) Van Herle, A. J., Uller, R. P., Matthews, N. L. & Josia, B.: Radioimmunoassay for measurement of thyroglobulin in human serum J. Clin. Invest., 52, 1320-1327, 1973.
7) Endo, Y., Nakano, J., Ohtaki, S., Izumi, M., Hamaguchi, Y., Yoshitake, S. & Ishikawa, E.: An Enzyme immunoassay for the measurement of thyrogulobulin in human serum. Clin. Acta., 95, 325-336, 1979.
8) Bayer, M. F. & Kriss, J. P.: Immunoradiometric assay for serum thyroglobulin in antithyroglobulin-positive sera. J. Clin. Endocrinol. Metab., 49, 557-564, 1979.
9) Kato, R., Noguchi, S. & Noguchi, A.: Human serum thyroglobulin determination with monoclonal antibody one-step assay: minimum interference of autoantibody. Endocrinol. Japan., 34(3), 171-178, 1987.
10) Sritongkul, N., Izumi, M., Kudo, I., Ohtakara, S., Ashizawa, K., Harakawa, S., Nagayama, Y., Yokoyama, N., Nanba, H., Kiriyama, K., Morimoto, I., Okamoto, S. & Nagataki, S.: The concentration of serum thyroglobulin (Tg) in antiTg auto-antibody (Ab) positive patients with Graves' disease. Vichayanrat, A., Nitiyanant, W., Eastman, C. & Nagataki, S. Recent progress in thyroidology, The third asia and oceania thyroid association meeting., 116-119, Crystal House Press, 1986.
11) Black, E. G., Cassoni, A., Gimlette, T. M. D., Harmer, C. L., Maisey, M. N. & Optes. G. D.: Serum thyroglobulin in thyroid cancer. Lancet., 29, 443-445, 1981.
12) Ericsson, U. B., Tegler, L., Lennquist, S., Christensen, S. B., Stahl, E. & Threll, J. I.: Serum thyroglobulin in differentiated thyroid carcinoma. Acta. Chir. Scand., 150, 367-375, 1984.
13) Sikorska, H. M.: Anti-thyroglobulin anti idiotypic antibodies in sera of patients with Hashimoto's thyroiditis and Graves' disease.

J. Immunol., 137, 3786-3795, 1987.
14) Derrin, D. R., Michel, R. & Roche, J.: Recherches sur la preperation et les proprietes de la thyroglobuline pure. I. Biochem. Biophys. Acta., 2, 454-470, 1948.
15) 岩崎辰夫, 安東民衛, 市川かおる, 保井孝太郎: 単クローン抗体, ハイブリドーマと ELISA. 50-82, 講談社サイエンティフィック, 1983.
16) Stephensen, J. R., Lee, J. M. & Wilton-Smith, P. D.: Production and purification of murine monoclonal antibodies: Aberrant elution from protein A-Sepharose 4B. Anal. Biochem. 142, 189-195, 1984.
17) Guesdon, J., Ternynck, T. & Avrameas, S.: The use of avidin-biotin interaction in immunoenzymatic technichs. J. Histochem. Cytochem., 27, 1131-1139, 1979.
18) Narkar, A. A., Shah, D. H., Swaroop, V. D., Velumani, A., Dan derkar. & Sharma, S. M.: Monoclonal antibodies to human thyroglobulin: Production and characterization. Hybridoma., 7, 97-104, 1988.
19) Marriotti, S., DeGroot, L. J., Scaborough, D. & Medof, M. E.: Study of circulating immune complexes in thyroid disease: Comparison of Raji cell radioimmunoassay and specific thyroglobulin-antithyroglobulin radioassay. J. Clin. Endoclinol. Meteb., 49, 679-686, 1979.
20) Ohtaki, S., Endo, Y., Horinouchi, K., Yoshitake, S. & Ishikawa, E.: Circulating thyroglobulin-antithyroglobulin immune complex in thyroid disease using enzyme-linked immunoassays. J. Clin. Endocrinol. Metab., 52, 239-245, 1981.
21) Marriotti, S., Cupini, C., Glani, C., Lari, R., Rollri, E., Falco, A., Marchisio, M. & Pinchera, A.: Evaluation of solid-phase immunoradiometric assay (IRMA) for serum thyroglobulin: effect of anti-thyroglobulin autoantibody. Clin. Chim. Acta., 123, 347-355, 1982.
22) Monaco, F., Grimaldi, S., Dominici, R. & Robbins, J.: Defective thyroglobulin synthesis in experimental rat thyroid tumor: Iodination and thyroid hormone synthesis isolated tumor thyroglobulin. Endocrinology., 97, 347-351, 1975.
23) Schneider, A. B., Ikekubo, K. & Kuma, K.: Iodine content of serum thyroglobulin in normal individuals and patients with thyroid tumors, J. Clin. Endocrinol. Metab., 57, 1251-1256, 1983.
24) Izumi, M., Cahnmann, H. J. & Robbins, J.: Characterization of abnormal thyroglobulin in transplantable rat thyroid tumor, Endocrinology., 100, 1448-1460, 1977.
25) Heilig, B., Hüfner, M., Dorken, B. & Schmidt-Gayk, H.: Increased heterogeneity of serum thyroglobulin in thyroid cancer patient as determined by monoclonal antibodies,, Kin Wochenschr., 64, 776-780, 1986.
26) Hüfner, M., Pfahl, H., Bethauser, H., Heilig, B. & Georg, P.: Comparative plasma throglobulin measurements with three non-cross-reactive monoclonal antibodies in metastatic thyroid cancer patients, Acta Endocrinol., 118, 528-532, 1988.
27) Kurata, A., Ohta, K., Mine, M., Fukuda, T., Ikari, N., Kanazawa, H., Matsunaga, M., Izumi, M. & Nagataki, S.: Monoclonal anti human thyroglobulin antibodies. J. Clin. Endoclinol. Metab., 59, 573-579, 1984.
28) Shepherd, P. S., Lazams, C. R., Mistry, R. D. & Maisey, M. N.: Detection of thyroid tumor using a monoclonal I-123-antihuman thyroglobulin antibody. Eur. J. Nucl. Med., 10, 291-295, 1985.
29) Jerne, N. K.: Towards a network theory of immune system. Ann. Immunol., 125c, 373-389, 1974.
30) Zouali, M., Fine, J-M. & Eyquem, A.: A human monoclonal IgG1 with anti idiotypic activity against anti-human thyroglobulin autoantibody. J. Immunol., 133, 190-194, 1984.
31) Hara, Y., Sridama, V. & DeGroot, L. J.: Auto-anti-idiotype antibody against antithyroglobulin auto-antibody in humans. J. Clin. Lab. Immunol., 26, 13-20, 1988.

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J SAITAMA MED SCH, (1989) 16 (3), 353-364

CANCER RESEARCH, (1975 Oct) 35 (10) 2689-92

KLINISCHE WOCHENSCHRIFT, (1982 May 3) 60 (9) 457-64

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Suppressive effect of prolactin on oestrogen-induced secretion of LH by sequentially perifused rat hypothalamus-pituitary

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Abstract. The effect of prolactin (Prl) on oestrogen-induced gonadotrophin secretion was examined in vitro in a sequential double chamber perifusion system. As control groups, mediobasal hypothalamus (MBH)-pituitary pairs or pituitaries without the MBHs were perifused with Medium 199. As an experimental group, MBH-pituitary pairs were perifused with Medium 199 containing 1 μg/ml of rat Prl. These groups were stimulated with 10^{-7} M oestradiol-17β (E_2) for 30 min, and luteinizing hormone (LH) in the serial fractions of effluent was measured.

In the control group of MBH-pituitary pairs perifused with medium without Prl, secretion of LH began to rise within 30 min after the beginning of stimulation, reached a peak 30 min after the end of stimulation and then remained at a plateau for the rest of the experimental period, whereas in the control group of pituitaries alone no significant response was observed. In the experimental group perifused with medium containing Prl, LH-secretion showed peaks 20 and 80 min after the end of E_2-stimulation, respectively, and the first peak was significantly ($P < 0.01$) less than the level in the control group.

These data demonstrate that Prl at this concentration suppressed the rapid LH release induced by E_2. Its site of action is suggested to be at the hypothalamic level, and its possible mechanism of action is discussed.

Possible mechanisms by which prolactin (Prl) suppresses gonadotrophin release reported by others are as follows: first, Prl may suppress hypothalamic luteinizing hormone releasing hormone (LRH) release (Gil-Ad et al. 1978; Grandison et al. 1977; Smith 1980). The mechanism of this action has been postulated by many investigators to be that increased Prl increases dopamine (DA) turn-over via a short loop feedback mechanism and the increased DA level, in turn, suppresses LRH release (Gudelsky et al. 1976; Chatani et al. 1983; Esquifino et al. 1984). Second, Prl can directly suppress the pituitary responsiveness to LRH in vivo (Vasquez et al. 1980; Carter & Whitehead 1981) and in vitro (Cheng 1983) by decreasing LRH receptors in the pituitary gland (Clayton & Bailey 1982; Marchetti & Labrie 1982).

On the other hand, there are several reports about the suppressive effect of Prl on oestrogen-induced gonadotrophin secretion in patients with the galactorrhoea-amenorrhoea syndrome (Aono et al. 1976), and drug-induced hyperprolactinaemia (L'Hermite et al. 1978; Anderson et al. 1982). restoration of oestrogen positive feedback by bromocriptine treatment (Aono et al. 1979) and by surgical removal of a pituitary adenoma (Koike et al. 1982) have also been reported.

However, the mechanism and site of action of Prl for this suppressive effect is uncertain. Therefore, we examined the mechanism of the suppressive effect of Prl on luteinizing hormone (LH) release in an in vitro perifusion system.

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Materials and Methods

Female Wistar-Imamichi rats (Nihon Laboanimal Co., Osaka, Japan) weighing 220–250 g in dioestrus were used. They were housed under controlled lighting conditions (lights on from 06.00 to 21.00 h) at 25°C and given free access to water and food. The rats were killed at 12.30 h and their MBH and/or pituitary was removed. The MBH and pituitary were placed in the first and second chamber, respectively, of a sequential double-chamber perifusion apparatus (Miyake et al. 1982). The MBH tissue block excised was demarcated by the hypothalamic sulci laterally, the caudal aspect of the optic chiasm rostrally and the rostral aspect of the mamillary bodies caudally. The whole pituitary glands were used without hemisection.

In the first study, 8 MBH-pituitary pairs in sequence were perifused with Medium 199 (Handai-Biken, Japan) containing antiserum to LRH obtained from a rabbit at 1:100 dilution or normal rabbit serum at the same dilution. In the second study, 6 MBH-pituitary pairs in sequence and 7 pituitaries without MBHs were perifused with Medium 199 as a control group. In an experimental group, 8 MBH-pituitary pairs were perifused with Medium 199 containing 1 μg/ml of rat prolactin (NIAMD-rPrl-B-3). Perifusion was started immediately after sacrifice at a flow rate of 3 ml/h with medium saturated with 95% O_2–5% CO_2 at 37°C. The perifusion system was equilibrated for 2.5 h and samples of 0.5 ml each were collected at 10 min interval from 15.00 h. Six samples were collected over a 1-h period, and then 10^{-7} M oestradiol-17β (E_2) in Medium 199 was perifused for the next 30 min. Eighteen fractions in one experiment were collected for 3 h and stored at −20°C until assayed. Rat LH in these fractions was measured by radioimmunoassay (Hayashi et al. 1976). The sensitivity of the LH assay and its intra-assay coefficient of variation were 2 ng NIADDKD-rat LH-RP-2/tube and 7.5%, respectively. In each experiment the mean concentration of LH in the 6 fractions collected during 1 h before each treatment was used as the basal value, and values during experiments were calculated as percentage changes from the mean basal value in each group. Two-way analysis of variance was used for evaluation of the statistical significances of differences in the stimulatory effects of oestrogen and suppressive effects of Prl, respectively.

Results

In the first experiment on MBH-pituitary pairs perifused with normal rabbit serum, LH release began to increase within 30 min after the beginning of E_2 stimulation ($P < 0.01$), reaching peak of 147.7% over the basal value 30 min after the beginning of E_2 stimulation ($P < 0.01$), and then undulating at the plateau level for the rest of the experimental period (Fig. 1). On the contrary, as shown in Fig. 1, no significant LH release after E_2 stimulation was observed in the group perifused with antiserum to LRH. LH changes in the second experiment using Prl are shown in Fig. 2. In the group of MBH-pituitary pairs without Prl, E_2 administration caused significant LH release (130.4% increase over the basal value) as observed in the first experiment of perifused MBH-pituitary pairs without antiserum to LRH. No significant change in LH release was observed with pituitaries alone. The second experiment of MBH-pituitary pairs perifused with Prl showed two peaks: a first lower peak of 47.8% increase over the basal value ($P < 0.01$) was noted 20 min after the end of E_2 stimulation, and a second higher peak of 71.5% increase over the basal value ($P < 0.01$) was seen 80 min after the end of stimulation. The first peak was significantly less ($P < 0.01$) than that of the control group, but the second peak was not significantly different from that of the control.

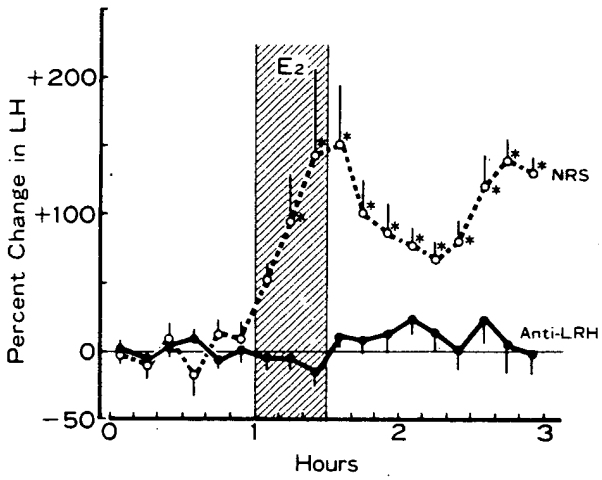

Fig. 1.
LH release from whole pituitary in sequence with MBH by E_2 perifused Medium 199 containing normal rabbit serum or Medium 199 containing antiserum to LRH. In the group perifused with Medium 199 containing normal rabbit serum, LH concentrations in the effluent increased significantly showing two peaks, while in the group perifused with antiserum to LRH no significant LH release was observed. * $P < 0.01$, basal vs E_2-stimulated.

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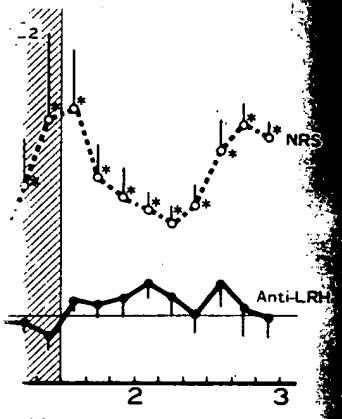

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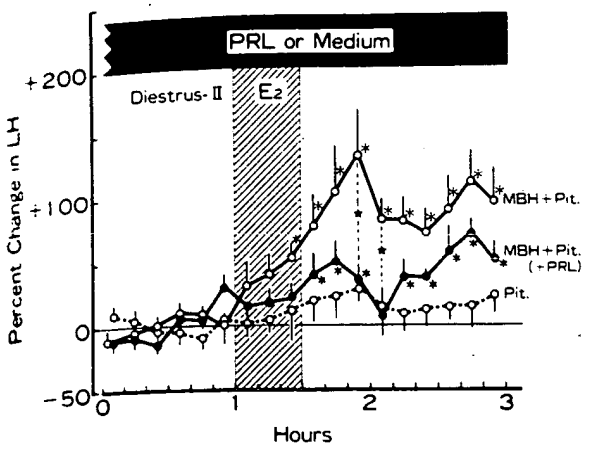

Fig. 2.

Rapid release of LH by E_2 and its suppression by Prl. In the control group without Prl, secretion of LH by MBH-pituitary pairs increased rapidly and reached a peak 30 min after the end of E_2-stimulation, whereas secretion of LH by pituitaries without the MBH was not affected by E_2. In the experimental group perifused with Prl the response was significantly less. 1) Basal vs E_2-stimulated $*P < 0.01$. 2) Control vs experimental $*P < 0.01$.

Discussion

In the present first study, LH secretion began to rise 30 min after the beginning of E_2 stimulation of MBH-pituitary pairs perifused with medium containing normal rabbit serum, but this rise was not observed under perifusion with antiserum to LRH. These results suggest that this LH secretion is probably due to stimulation by E_2 of LRH release from the MBH.

Although the site of the inhibitory effect of E_2 on LH release has been reported to be at the pituitary gland in vivo (Negro-Vilar et al. 1973), the acute inhibition of LH release from the pituitary gland by E_2 has been observed in vitro neither in the previous studies (Turgeon & Waring 1981; Miyake et al. 1982) nor in the present study. The mechanism of difference in LH release following E_2 administration between in vivo and in vitro studies is not clear at present.

An important finding in the present study was the rapidity of the LH response to oestrogen i.e., LH started to rise within 30 min after stimulation. This suggests that the LRH was 'released' rather than newly 'synthesized', since a period of 30 min was too short to be compatible with the time needed for synthesis of the peptide (McEwen et al. 1982; Drouva et al. 1984).

Synaptosomal release of LRH without cell bodies has been mentioned in serveral reports (Tytell et al. 1980; Dever et al. 1980; Warberg 1982; Drouva et al. 1984). Drouva et al. (1984) reported that 1) E_2 appears to be selectively and specifically involved in process coupling, nerve ending depolarization and, in turn, LRH release, and 2) the effect of E_2 is receptor-mediated and does not appear to require nuclear translocation of the steroid or transcription procedures, since it can be readily elicited upon simple addition of E_2 to nerve endings disconnected from their cell bodies. Furthermore, Warberg (1982) found that LRH, TRH and α-MSH are concentrated in synaptosome-riched fractions where they are present in granules, and that they are released in a Ca^{++}-dependent manner by stimuli considered to depolarize the neural membranes. In addition, the depolarization procedure in synaptic transmission shows only 0.5 msec of 'synaptic delay' (Berne & Levy 1983). Consequently this LRH release is concluded not to result from new synthesis of LRH but rapid and direct release of LRH from stores, as in the synaptosomal experiments described above.

In the present study, Prl suppressed oestrogen-induced LH release only partially. For the effect of Prl, its concentration and its duration of reaction are important (Cheng 1983), because the suppressive effect of Prl is dose-dependent. In the present experiment, we added Prl at 1 μg/ml, which corresponds to about the upper level in severe hyperprolactinaemia in humans. This concentration may be relatively low considering that we examined its effect in vitro, and that the perifusion time before E_2 stimulation was 3 h. However, although the concentration of Prl and the duration of treatment in the present experiment may not have been sufficient to cause complete suppression, we observed significant suppression of the first peak.

Prl is known to suppress gonadotrophin secretion by increasing DA turn-over (Gudelsky et al. 1976; Esquifino et al. 1984; Chatani et al. 1983), but this mechanism does not seem to explain the present results adequately. Moreover, this process has been noted to be very slow, being apparent about 12–16 h after increase of Prl (Moore et al. 1980), and so it is unlikely that DA increase by short-loop feedback of Prl control operates during

a short period of within 1 h. Thus in our experiment Prl may have had a direct suppressive effect on the responsiveness of the pituitary to LRH.

In the present experiment, the pituitary without MBH did not respond to oestrogen and was therefore not perifused with Prl. But a direct suppressive effect on the MBH cannot be disregarded, because gonadotrophin secretion is the result of a response of the pituitary to LRH. Indeed there are many reports of this direct suppressive effect both in vivo (Vasquez et al. 1980; Carter & Whitehead 1981) and in vitro (Cheng 1983). However, the time sequences of results in these reports were very different from that in the present experiment. Furthermore, as mentioned above, we used a concentration of Prl of less than one twentieth of that used in a previous in vitro study on the pituitary without the MBH (Cheng 1983). In that experiment, Prl at 20 μg/ml significantly suppressed the response of the pituitary gland to LRH. Therefore, caution is required in concluding that this direct suppression did not occur in the present experiment.

From the above considerations, and since there was not other possible influencing factor and the reaction time was very short, it appears likely from the present experiment that Prl directly inhibited the oestrogen-induced gonadotrophin releasing mechanism at the hypothalamic level. Further studies seem necessary on the effect of Prl on LRH and DA releases in longer observation periods.

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References

Anderson A N, Schoeler V, Hertz J & Bennett P (1982): Effect of metoclopramide induced hyperprolactinaemia on the gonadotrophic response to oestradiol and LRH. Acta Endocrinol (Copenh) 100: 1–9.

Aono T, Miyake A, Shioji T, Kinugasa T, Onishi T & Kurachi K (1976): Impaired LH release following exogenous estrogen administration in patients with the amenorrhea-galactorrhea syndrome. J Clin Endocrinol Metab 42: 696–702.

Aono T, Miyake A, Shioji T, Yasuda M, Koike K & Kurachi K (1979): Restoration of oestrogen positive feedback effect of LH release by bromocriptine in hyperprolactinaemic patients with galactorrhoea-amenorrhoea. Acta Endocrinol (Copenh) 91: 591–600.

Berne R M & Levy M N (1983): Synaptic transmission. In: Harchbergen S E (ed). Physiology. Mosby Co.

Carter D A & Whitehead S A (1981): Pituitary sensitivity to gonadotrophin releasing hormone after hyperprolactinemia induced with domperidone in the rat. J Endocrinol 91: 439–446.

Chatani F, Aono T, Koike K, Tasaka K & Kurachi K (1983): Effect of sulpiride-induced hyperprolactinaemia on catecholamine turn-over and LRH concentration in the medial basal hypothalamus of rats. Acta Endocrinol (Copenh) 102: 321–326.

Cheng C Y (1983): Prolactin suppresses luteinizing hormone secretion and pituitary responsiveness to luteinizing hormone-releasing hormone by a direct action at the anterior pituitary. Endocrinology 113: 632–638.

Clayton R N & Bailey L C (1982): Hyperprolactinemia attenuates the gonadotropin releasing hormone receptor response to gonadectomy in rats. J Endocrinol 95: 267–274.

Deyer R G, Mansfield S & Yates J O (1980): Discharge of gonadotropin-releasing hormone from the mediobasal part of the hypothalamus: effect of stimulation frequency and gonadal steroids. Exp Brain Res 39: 453–460.

Drouva S V, Laplante E, Gantron J P & Kordon C (1984): Effect of 17β-oestradiol on LHRH release from rat mediobasal hypothalamic slices. Neuroendocrinology 38: 152–157.

Esquifino A I, Ramos J A & Treguerres J A F (1984): Possible role of dopamine in changes in LH and prolactin concentrations after experimentally induced hyperprolactinemia in rats. J Endocrinol 100: 141–148.

Gil-Ad I, Locatelli V, Cocchi D, Carminati R, Arezzooini C & Mueller E E (1978): Effect of hyperprolactinemia and 2-BR-α-ergocryptine on neuroendocrine mechanism for gonadotropin control. Life Sci 23: 2245–2256.

Grandison L, Hodson C, Chen H T, Davis J, Simpkins J & Meites J (1977): Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology 23: 312–322.

Gudelsky G A, Simpkins J, Mueller G P, Meites J & Moore K E (1976): Selective action of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 22: 206–215.

Hayashi T, Glotz H J, Aono T & Matsumoto K (1976): Influence of gestonorone caproate on rat prostate. J Steroid Biochem 11: 1217–1221.

Koike K, Aono T, Tsutsumi H, Miyake A & Kurachi K

(1982): Restoration of oestrogen positive feedback effect on LH release in women with prolactinaemia by transsphenoidal surgery. Acta Endocrinol (Copenh) 100: 492–498.

L'Hermite M, Delogne-Desnoeck J, Michaux-Duchene A & Robyn C (1978): Alteration of feed-back mechanism of estrogen on gonadotropin by sulpiride-induced hyperprolactinemia. J Clin Endocrinol Metab 47: 1132–1136.

Marchetti I & Labrie F (1982): Prolactin inhibits pituitary luteinizing hormone-releasing hormone receptor in the rat. Endocrinology 111: 1209–1216.

McEwen B A, Bigeon A, Davis P G, Krey L C, Luine V N, McGinnis M Y, Paden C M, Parsons B & Rainbow T C (1982): Steroid hormones: hormonal signals which alter brain cell properties and functions. Recent Prog Horm Res 38: 41–92.

Miyake A, Tasaka K, Kawamura Y, Sakumoto T & Aono T (1982): Progesterone facilitates the LRH releasing action of oestrogen. Acta Endocrinol (Copenh) 101: 321–324.

Moore K E, Demarest K T & Johnstone C A (1980): Influence of prolactin on the dopaminergic neuronal system in the hypothalamus. Fed Proc 39: 2912–2916.

Negro-Villar A, Orias R & McCann S M (1973): Evidence for a pituitary site of action for the acute inhibition of LH release by estrogen in the rat. Endocrinology 92: 1680–1684.

Smith M S (1980): Role of prolactin regulating gonadotropin secretion and gonadal function in female rats. Fed Proc 39: 2571–2576.

Turgeon J L & Waring D W (1981): Acute progesterone and 17β-estradiol modulation of luteinizing hormone secretion by pituitaries of cycling rats superfused in vitro. Endocrinology 108: 413–419.

Tytell M, Clark J H & Peck J H Jr (1980): Properties of LHRH release from a hypothalamic synaptosomal fraction of estrogen-primed ovariectomized rats. Neurochem Res 5: 479–491.

Vasquez J M, Nazian S T & Mahesh V B (1980): Pituitary sensitivity to LHRH in hyperprolactinemia induced by perphenzine and renal pituitary transplants in female rats. Biol Reprod 22: 486–492.

Warberg J (1982): Studies on the release mechanism for hypothalamic hormone. Acta Endocrinol (Copenh), Suppl 250, 101: 1–46.

Received on April 17th, 1984.

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ACTA ENDOCRINOLOGICA, (1985) Vol. 108, pp. 151

Eur J Nucl Med, (1981). Vol. 6, No. 11, pp. 515-520

ENDOCRINOL SUPPL. (1985) 108 (267), 151

J SAITAMA MED SCH, (1989) 16 (3), 353-364

CANCER RESEARCH, (1975 Oct) 35 (10) 2689-92

KLINISCHE WOCHENSCHRIFT, (1982 May 3) 60 (9) 457-64

CHINESE MEDICAL JOURNAL, (1989 Apr) 102 (4) 282-9

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION, (1990 Oct) 13 (9) 737-42

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[CANCER RESEARCH 35, 2689-2692, October 1975]

Carcinoembryonic Antigen and Humoral Antibody Resp nse in Patients with Thyroid Carcinoma[1]

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SUMMARY

Carcinoembryonic antigen and antibodies to thyroglobulin and to a microsomal fraction of thyroid were measured. Persons examined were normal volunteers, patients with thyroid cancer, and patients with a history of childhood irradiation to the thymus and/or tonsil who were otherwise normal. Elevated antigen and antibodies were most frequently found in the cancer thyroid group. Thyroid cancer patients with no previous history of childhood irradiation were more frequently positive for antigen and antibodies than all other categories studied. Thyroid cancer patients with a previous history of childhood irradiation showed normal frequencies of antigen and antibodies. The results suggest that the antigenic expression and host response to the tumor in patients with thyroid cancer depend on its pathogenesis. Mention is made of similar findings in animal model systems.

INTRODUCTION

Elevated levels of circulating CEA[3] have been found in association with various cancers, especially those originating from the gastrointestinal tract (6, 13). Less frequently, elevated levels have also been obtained with certain nongastrointestinal cancers, such as those involving the breast, bronchus, and prostate (5, 9).

The only reported study of CEA levels in patients with thyroid disease that the authors are aware of is that of Laurence *et al.* (5). They studied 6 patients with nodular goiter, 2 with adenomata, and 1 with a carcinoma. None of their patients had elevated levels. The present investigation was undertaken to determine the usefulness of measuring circulating CEA in patients with thyroid cancer. Their humoral antibody response to a microsomal fraction (of thyroid) and to thyroglobulin was also examined. Irradiation to the thymus or tonsillar area in infancy has been shown to result in an increased incidence of subsequently developing thyroid cancer (4). Therefore, in this study, the results of assays on patients were analyzed to determine whether this factor (irradiation) had any influence on the data that were obtained.

MATERIALS AND METHODS

Controls. Twenty-nine subjects consisting of volunteer students and technicians, all nonsmokers, served as controls for the CEA assay. One hundred unselected preemployment personnel served as controls for the antibody tests.

Patients. In all, 237 patients were studied. Fifty-seven of these patients had thyroid cancer, of whom 26 had no previous history of childhood irradiation to the tonsil and/or thymic areas, and their mean age was 38.4 ± 14.7 years (S.D.). The remaining 31 thyroid cancer patients (mean age, 30.7 ± 10.1 years) had a history of childhood irradiation to the thymus or tonsil, and this was corroborated by a hospital record in 63% of cases. One hundred eighty patients, otherwise normal, but with a history of irradiation to the thymus or tonsil and who sought medical advice to exclude pathology, were studied; the history of 53% of these patients was corroborated by a hospital record.

A careful history was obtained and a full physical examination was carried out on all patients. Special investigations done included serum total thyroxine, radioactive iodine uptake, and thyroid scan.

The procedure for measuring CEA was that as described by Laurence *et al.* (5), which is a modification of the triple-isotope method of Egan *et al.* (3). (CEA and its antibody were gifts from Dr. Charles W. Todd, Department of Immunology, City of Hope National Medical Center, Duarte, California 91010.) In this assay system, levels greater than 12.5 ng/ml are considered abnormal, although not exclusively due to the presence of cancer.

Thyroglobulin antibodies were measured with the Thyroid Test Kit and microsomal antibodies were measured with the Microsome Test Kit, both obtained from Fujizoki Pharmaceutical Co., Ltd., Tokyo, Japan. A positive reaction at a serum dilution of 1:20 or greater was considered indicative of the presence of antibody.

RESULTS

Levels of circulating CEA in volunteers and patients are shown in Tables 1 and 4. Elevated CEA levels were found in 24% of noncancer patients with a history of childhood irradiation, compared to 10% for the control group. The increased frequency of elevated CEA levels in the noncancer

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[3] The abbreviation used is: CEA, carcinoembryonic antigen.

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patients is difficult to explain. One contributing factor may have been the inclusion of smokers or those wh had smoked (40% of these patients), since moderate to heavy smoking is frequently associated with an elevated CEA level (10, 12), and this was also found in the present study. In the thyroid cancer group, 36% had elevated CEA levels. Analysis of the cancer patients showed 18% (3 of 17) of those with a previous history of irradiation to have an elevated CEA level; this compared with 56% (9 of 16) in the group of thyroid cancer patients with no known previous history of irradiation to the tonsil or thymus. Furthermore, in this latter group, when the CEA level was elevated, the concentration frequently tended to be higher (>20 ng/ml) than that in all other categories of patients. The results of examining volunteers and patients for thyroglobulin and microsomal antibodies are shown in Tables 2 to 4. Thyroglobulin antibodies were detected more frequently in patients with thyroid cancer and this was solely due to those without a history f irradiation exposure (Tables 2 and 4). Similarly, microsomal antibodies were more frequently detected in the thyroid cancer group and this also was solely due to those without a history f irradiation exposure (Tables 3 and 4).

No obvious relationship was found between the levels of CEA and the spread or grade of tumor involved. Similarly, the antibody titers did not correlate with the degree of tumor spread or differentiation.

DISCUSSION

CEA levels were more frequently elevated in noncancer patients than in control subjects. This may have been due to

Table 1

Circulating CEA levels (postoperative) in patients with cancer of the thyroid

	No. of subjects	No. of subjects with following CEA concentration			% positive	χ^2 test
		<12.5 ng/ml	12.5–20 ng/ml	>20 ng/ml		
Control group (students and technicians; nonsmokers)	29	26	3		10	
History of childhood irradiation to thymic or tonsillar region; examination revealed no obvious pathology	105	80	24	1	24	NS[a]
Carcinoma of the thyroid	33	21	9	3	36	$p < 0.02$
In patients with a previous history of childhood irradiation	17	14	3		18	NS
No previous history of irradiation	16	7	6	3	56	$p < 0.01$

[a] NS, not significant.

Table 2

Circulating thyroglobulin antibody levels (postoperative) in patients with cancer of the thyroid

	No. of subjects	Thyroglobulin antibodies				χ^2 test
		Not detected	Detected		% positive	
			Titer	No. positive		
Unselected preemployment personnel	100			3	3	
History of childhood irradiation to thymic or tonsillar region; examination revealed no obvious pathology	119	110	1/20 1/40 1/80 1/320	4 2 2 1 } 9	8	NS[a]
Cancer of the thyroid	51	44		7	14	$p < 0.02$
In patients with a previous history of childhood irradiation	31	30	1/20	1	3	NS
No previous history of childhood irradiation	20	14	1/20 1/40 1/160 1/640	2 2 1 1 } 6	30	$p < 0.01$

[a] NS, not significant.

Table 3

Circulating microsomal antibody levels (postoperative) in patients with cancer of the thyroid

		Microsomal antibodies				
			Detected			
	No. of subjects	Not detected	Titer	No. positive	% positive	χ^2 test
Unselected preemployment personnel	100			10	10	
History of childhood irradiation to thymic or tonsilar region examination revealed no obvious pathology	111	92	1/20	3		
			1/320	1		
			1/640	3		
			1/1,280	7	19	17 NS[a]
			1/2,560	3		
			1/10,240	2		
Cancer of the thyroid	37	29		8	22	$p < 0.10$
In patients with a previous history of childhood irradiation	18	16	1/80	1	2	11 NS
			1/160	1		
No previous history of childhood irradiation	19	13	1/80	3		
			1/320	2	6	32 $p < 0.02$
			Not titrated	1		

[a] NS, not significant.

Table 4

Antigen and antibody in thyroid cancer patients

	Patients with no previous history of childhood irradiation		Patients with a previous history of childhood irradiation		
Antigen or antibody	No. of patients	% positive	No. of patients	% positive	χ^2 test
CEA	16	56	17	18	$p < 0.05$
Thyroglobulin antibodies	20	30	31	3	$p < 0.01$
Microsomal antibodies	19	32	18	11	NS

the contribution of patients with smoking habits, many of whom had CEA levels in the range of 12 to 20 ng/ml. Patients with a history of irradiation to the tonsil or thymic area with or without thyroid cancer had comparable levels of CEA (18 and 24%). In contrast, thyroid cancer patients without a previous history of childhood irradiation had more frequently an associated elevation in the CEA (56% positive). This finding is consistent with the results of other investigators who studied transplantation antigens. Unlike the tumor transplantation antigens, CEA is not immunogenic in the autochthonous host. Nevertheless, present evidence suggests that CEA, α-fetoprotein, and the chemically induced cancer transplantation antigens are all embryonic in origin, being reexpressed with tumorigenesis (1). Moore and Williams (8) have demonstrated that most murine irradiation-induced osteosarcomata have a paucity of tumor-specific cell surface antigens. Furthermore, they found that osteosarcomata induced by a chemical carcinogen differs significantly in antig nic strength fr m th se induced by irradiation (^{32}P), and concluded that, in these contrasting models of bone onc genesis, antigenicity was more a function of the carcinogenic agent than the site of tumor origin. Similarly, Stjernswärd (11) found that irradiation-induced osteosarcomata was associated with a lower degree of antigenicity, compared with some (but not all) osteosarcomata induced by chemical carcinogens. Baldwin *et al.* (1) also reached a similar conclusion when studying irradiation- and chemically induced osteosarcomata.

A positive correlation has been found between the spread of tumor in cancer of the colon and CEA levels (13). However, in the present study no such relationship could be found. Published reports are not consistent regarding a relationship between the degree of tumor differentiation and CEA levels. Denk *et al.* (2) found that the more differentiated tumors had a greater abundance of CEA. In contrast, Martin and Martin (7) found no obvious correlation. Both studies were concerned with gastrointestinal cancer. In the present investigation, there was no evidence of CEA levels being related to the degree of tumor differentiation. The types of tumor present (not shown) in both groups of thyroid patients were essentially similar; almost all were of the well-differentiated type. There was no correlation of age with the CEA level, and this confirms the results of previous reports.

In addition to the findings in this study of an association of thyroid cancer with CEA levels, a similar tendency was also noted in the antibody studies. Those thyroid cancer patients without a history of irradiation to the thymus or tonsil more frequently were found to have antibodies directed against thyroglobulin and to a lesser extent against the microsomal fraction of thyroid (Tables 3 and 4). These findings are consistent with studies in laboratory animals; Stjernswärd (11) found the antibody response to an injected antigen (sheep red blood cells) to be generally less in irradiati n-induced osteosarcomata than that induced by a chemical carcinogen.

The present study is in accord with the findings in experimental model systems and suggests that antigenic expression and host response to the tumor in patients with thyroid cancer depend on its pathogenesis.

ACKNOWLEDGMENTS

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REFERENCES

1. Baldwin, R. W., Embleton, M. J., Price, M. R., and Vose, B. M. Embryonic Antigen Expression on Experimental Rat Tumors. Transplant. Rev., *20:* 77–99, 1974.
2. Denk, H., Tappeiner, G., Eckerstorfer, R., and Holzner, J. H. Carcinoembryonic Antigen (CEA) in Gastro-Intestinal and Extra-Gastro-Intestinal Tumors and Its Relationship to Tumor-Cell Differentiation. Intern. J. Cancer, *10:* 262–272, 1972.
3. Egan, M. L., Lautenschleger, J. T., Coligan, J. E., and Todd, C. W. Radioimmune Assay of Carcinoembryonic Antigen. Immunochemistry, *9:* 289–299, 1972.
4. Hempelman, L. H. Risk of Thyroid Neoplasms after Irradiation in Childhood. Science, *160:* 159–163, 1968.
5. Laurence, D. J. R., Stevens, U., Bettelheim, R., Darcy, D., Leese, C., Turberville, C., Alexander, P., Johns, E. W., and Neville, A. M. Role of Plasma Carcinoembryonic Antigen in Diagnosis of Gastrointestinal, Mammary and Bronchial Carcinoma. Brit. Med. J., *3:* 605–609, 1972.
6. LoGerfo, P., Krupey, J., and Hansen, H. J. Demonstration of an Antigen Common to Serial Varieties of Neoplasia. New Engl. J. Med., *285:* 138–141, 1971.
7. Martin, F., and Martin, M. S. Demonstration of Antigens Related to Colonic Cancer in the Human Digestive System. Intern. J. Cancer., *6:* 352–360, 1970.
8. Moore, M., and Williams, D. E. Studies on the Antigenicity of Radiation-induced Murine Osteosarcomata. Brit. J. Cancer, *26:* 90–98, 1972.
9. Reynoso, G., Chu, T. H., Holyoke, D., Cohen, E., Nemoto, T., Wang, J-J., Chuang, J., Guinan, P., and Murphy, G. P. Carcinoembryonic Antigen in Patients with Different Cancers. J. Am. Med. Assoc., *220:* 361–365, 1972.
10. Stevens, D. P., and Mackay, I. R. Increased Carcinoembryonic Antigen in Heavy Smokers. Lancet, *2:* 1238–1239, 1973.
11. Stjernswärd, J. Immunosuppression by Carcinogens. Antibiot. Chemotherapia, *15:* 213–233, 1969.
12. Terry, W. D., Henkart, P. A., Coligan, J. E., and Todd, C. W. Tumor Associated Embryonic Antigens. Transplant. Rev., *20:* 100–129, 1974.
13. Zamchek, N., Moore, T. L., Dhar, P., and Kupchik, H. Immunologic Diagnosis and Prognosis of Human Digestive-Tract Cancer: Carcinoembryonic Antigens. New Engl. J. Med., *286:* 83–86, 1972.

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ACTA ENDOCRINOLOGICA, (1985) Vol. 108, pp. 151

Eur J Nucl Med, (1981). Vol. 6, No. 11, pp. 515-520

ENDOCRINOL SUPPL. (1985) 108 (267), 151

J SAITAMA MED SCH, (1989) 16 (3), 353-364

CANCER RESEARCH, (1975 Oct) 35 (10) 2689-92

KLINISCHE WOCHENSCHRIFT, (1982 May 3) 60 (9) 457-64

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Usefulness of the combined antithyroglobulin antibodies and thyroglobulin assay in the follow-up of patients with differentiated thyroid cancer

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ABSTRACT. A total of 1050 patients with differentiated thyroid cancer (DTC) have been followed in the Thyroid Center of Padua by means of serum thyroglobulin (Tg) measured with IRMA method and anti-Tg antibodies (TgAb) assays. Circulating TgAbs were detected in 102 (9.7%) patients. In 32 of these 102, TgAbs were evaluated before and after total thyroidectomy and ^{131}I ablation. In these patients no relationship was found between preoperative serum TgAb levels on the one hand and tumor stage at diagnosis or outcome of the disease on the other. During the follow-up, TgAb serum levels decreased or disappeared in 21 cases considered tumor-free, while they remained unchanged or even increased, in comparison with the preoperative ones, in 11 patients, 5 with proven metastases and 6 considered tumor-free. Evaluating the whole group of 102 TgAb-positive patients, we observed that TgAb serum levels, measured after thyroid ablation, were significantly higher in cases with metastases than in those considered tumor-free (653.0 ± 196.9 vs 157.7 ± 116.5 U/ml, m ± SD, $p < 0.0001$). In the group of patients with metastases and circulating TgAbs, Tg serum levels were elevated in 27% of cases on TSH- suppressive therapy and in 44% off therapy when nodal metastases were present, and in 67% of cases on TSH-suppressive therapy and in 83% off therapy when distant metastases were present. Our data suggest that: i) In a very large series of patients with DTC, circulating TgAbs are detectable in 9.7% of cases; ii) In the follow-up, patients with high TgAb serum levels even in absence of detectable serum Tg values are at risk for metastases; in fact circulating TgAbs might be due to the presence of tumor and circulating TgAbs themselves may prevent the detection of serum Tg; iii) serum IRMA-Tg assay appears to maintain its value as a tumoral marker in more than half the patients with metastases and circulating TgAbs.

INTRODUCTION

A number of papers in literature show that serum Tg measurements, together with ^{131}I-total body scan (TBS), represent the most important methods in the search for recurrences or metastases in patients with differentiated thyroid cancer (DTC) (1-15). However, it is also known that TgAbs may interfere in Tg assay (16-22). So, in many previous studies on the value of serum Tg assay as a tumoral marker, patients with circulating TgAbs were excluded. Using radioimmunologic (RIA) assay, circulating TgAbs may induce either falsely elevated or depressed Tg values, whereas using immunoradiometric (IRMA) assay, the presence of TgAbs invariably results ir decreased Tg levels (16, 18, 19, 22).

Therefore, using IRMA methods, low serum Tg values in patients who had undergone thyroid ablation for DTC with circulating TgAbs, may be due either to the absence (true negative) or even to the presence (false negative) of thyroid tumor. On the other hand, it may be that in these TgAb-positive patients, elevated Tg serum levels maintain their significance as tumoral marker. The aims of the present study were: i) To ascertain the validity of

Key-words: Serum thyroglobulin, antithyroglobulin antibodies, thyroid cancer.

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Table 1 - *Percentage of patients with Tg serum levels > 3 ng/ml, on and off hormonal therapy, in cases with metastases and absence or presence of circulating TgAbs (cut off limit of 50 U/ml).*

METASTASES	NODES		LUNG		BONE	
N. CASES	74		21		24	
HORMONAL THERAPY	ON	OFF	ON	OFF	ON	OFF
Tg > 3; TgAb < 50	64%	93%	94%	94%	96%	100%
Tg > 3; TgAb > 50	27%	44%	50%	75%	100%	100%

the above assumption; ii) To ascertain the incidence of circulating TgAbs in a very large group of patients with DTC; iii) To verify the possible existence of a relationship between TgAb serum levels and the course of the disease.

MATERIALS AND METHODS

From 1967 to 1987 a total of 1457 patients with DCT were followed at the Thyroid Center of Padua and in 1050 their sera were analyzed in the Tg and TgAb assays.

TgAbs were detectable in 102 cases (9.7%): 84 females and 18 males, age ranged between 17 and 72 yr, mean 45.8. The histological examination showed papillary cancer in 80 cases and follicular in 22. Stages (TNM UICC, 1979) were as follows: T1-3NOMO in 35 patients, T1-3N1-2MO in 45, T1-4N1-3MO in 12 and M1 in 10. In 70 of these 102 patients the study was performed at least two years after treatment, whereas in 32 serum Tg and TgAb levels were evaluated both before and after treatment. Twenty six of them had papillary, 6 follicular tumor. Stages were as follows: T1-3NOMO in 10 patients, T1-3N1-2MO in 15, T1-4N1-3MO in 4, M1 in 3.

From the therapeutic point of view, all the 102 patients studied were treated by total thyroidectomy and radioiodine therapy. Then all received TSH-suppressive therapy with L-thyroxine and the adequancy of the treatment was periodically checked by 200 μg iv TRH stimulation (24) or, recently, by TSH IRMA assay. Follow-up varied from 2 to 10 yr, median 4.8.

To evaluate the sensitivity of serum Tg as tumoral marker on and off hormonal therapy in absence of circulating TgAbs we considered a group of 102 patients with metastases (Table 1).

Tg serum levels were assayed by IRMA method (HTGK-Sorin, Italy). The interassay variation coefficient (VC) was 6.5%, the intraassay VC was 3.1%. The cut-off limit to distinguish pathological from non pathological values was 3 ng/ml. Tg serum levels were measured during TSH-suppressive therapy and, subsequently, (i.e., within 3 months) 15 days after L-triiodothyronine withdrawal prior to a TBS.

TgAb serum levels were assayed initially (before the year 1980) by means of a semi-quantitative method, i.e. hemoagglutination technique (Wellcome, UK) and after the year 1980 by means of a RIA method (Biodata, Italy). The results reported in the present study, only are concerned with the data obtained in patients in whom TgAbs were measured, both in fresh and stored sera, by RIA method. The interassay VC of the RIA method for TgAbs was 6.7% and the intraassay VC was 6.3%. We considered TgAb values below 50 U/ml as negative.

The schedule used was: when first seen, both Tg and TgAb serum levels are measured. In TgAb-positive patients, TgAb serum levels are measured in parallel with Tg serum assay, whereas in TgAb-negative patients, TgAb levels are measured every two years.

Total triiodothyronine was assayed by RIA (Mallinckrodt, West Germany), normal values 80-200 ng/dl, total thyroxine by fluorescence polarization immunoassay (TDx-Abbott, USA), normal values 5-12 μg/dl, free thyroxine by RIA (Biorad, USA), normal values 0.8-2.3 ng/dl, TSH by IRMA (CIS, France), normal values 0.2-4 μU/ml.

Statistical analysis was performed using both paired and unpaired Student's *t* test; $p < 0.05$ was considered significant. Data are expressed as the mean $\pm$ SD.

RESULTS

In our series of 1050 patients with DCT, circulating TgAbs were detectable in 102 patients, i.e. 9.7% of the whole group evaluated.

Group of 32 TgAb-positive patients studied before and after treatment.

Before surgery, TgAbs were present in 31 cases

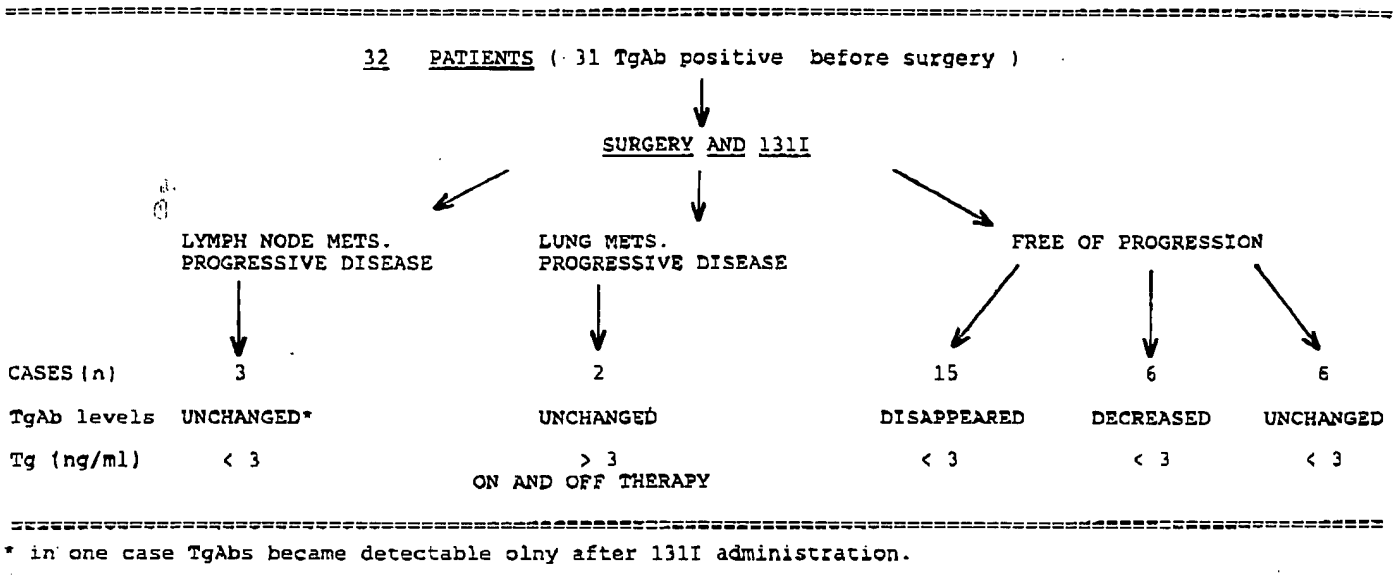

Fig. 1 - *Data concerning the group of 32 TgAb-positive patients studied before and after thyroid ablation.*

(394.9 ± 119.6 U/ml). In this group three patients with lymph node metastases and two with lung metastases at diagnosis showed a relapse or progression of disease: in 4 of them, TgAb serum levels remained high throughout the study, in the fifth case (lymph node metastasis) TgAbs became detectable only during the follow-up, and remained elevated during the study (TgAb serum levels were 340, 520, 630, 710 and 830 U/ml, respectively, at last control).

Tg serum levels were above the cut-off limit both on and off TSH-suppressive therapy only in the two patients with lung metastases (24 ng/ml on therapy and 410 ng/ml off therapy in one case and 810 ng/ml on therapy and 1290 ng/ml off therapy in the other case, respectively). The remaining 27 patients stayed free of tumor after treatment: in 15 of them (mean pre-surgical TgAb serum levels were 406.6 ± 189.0 U/ml) TgAb serum levels became undetectable within one year from therapy; in 6 cases TgAb serum levels decreased but remained positive (381.8 ± 203.9 vs 159.5 ± 86.9 U/ml, $p < 0.0001$); in 6 cases TgAb serum levels remained unchanged in comparison with the preoperative ones (464.8 ± 196.8 vs 398.2 ± 197.3 U/ml, p = NS). Tg serum levels were lower than 3 ng/ml in all 27 cases considered tumor-free both on and off TSH-suppressive therapy. No relationship was found between preoperative TgAb serum levels and tumor stage at diagnosis or disease outcome after therapy. The data concerning these 32 patients are resumed in Figure 1.

Group of 70 patients with circulating TgAbs, studied only after treatment.

Twelve patients had metastases: in 5 of them (3 nodal, 2 bone) Tg serum levels were elevated on and off TSH-suppressive therapy, while in another 3 cases (2 nodal, 1 lung) Tg serum levels increased only after thyroid hormone withdrawal. In the 58 patients considered free of disease, Tg serum levels were lower than 3 ng/ml both on and off thyroid hormone therapy.

Evaluating *the whole group of 102 TgAb-positive patients in the follow-up*,

a) Tg serum levels were high in 27% of patients with nodal and in 66% of those with distant metastases on therapy, and in 44% of cases with nodal and in 83% of those with distant metastases off therapy (Fig. 2),

b) TgAb serum levels were significantly higher in patients with recurrence or progression of disease than in patients tumor-free (653.0 ± 196.9 vs 157.7 ± 116.5 U/ml, $p < 0.0001$) (Fig. 3). Moreover, Figure 3 shows that TgAb values less than 400 U/ml are rarely associated with metastatic disease whereas values more than 400 U/ml are in 88% of cases associated with the presence of metastases.

Tg levels in patients with metastases and with or without TgAbs.

The sensitivity of serum Tg as tumoral marker for DTC was investigated comparing two groups of patients who were previously treated by total thyroidectomy and with known metastases, i.e. those with (17 cases) versus those without circulating

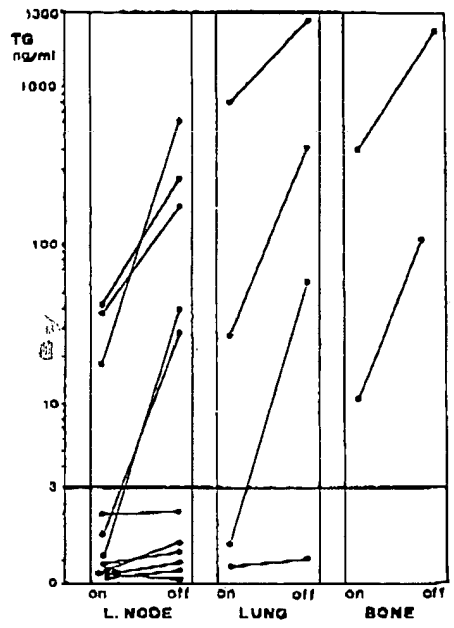

Fig. 2 - *Tg serum levels on and off TSH suppressive therapy in the group of 17 patients with lymph node (L. NODE), bone, lung metastases of differentiated thyroid cancer and circulating TgAb.*

TgAbs (102 cases). Table 1 shows the percentage of cases with increased Tg serum levels, on and off hormonal therapy, in the two groups of patients evaluated.

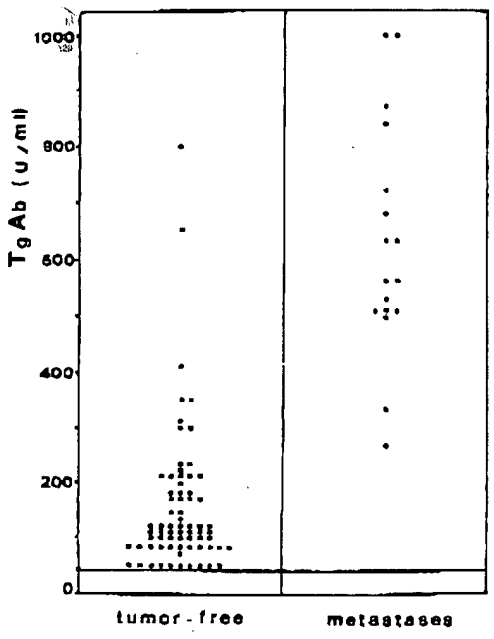

Fig. 3 - *TgAb serum levels measured in patients tumor-free (left panel) in comparison with those of patients with metastases (right panel).*

DISCUSSION

Serum Tg assay and ^{131}I-TBS represent the two main tools in the follow-up of patients with DTC after total thyroidectomy. In previous studies (10-12), we reported that ^{131}I-TBS is able to detect 78% of metastases (69% of nodal and 84% of distant). At the same time, in patients without circulating TgAbs, serum Tg determination was shown to be able to detect 64% of nodal and 93% of distant metastases, respectively, on TSH-suppressive therapy, and 95% of nodal and 98% of distant metastases, respectively, after thyroid hormone withdrawal.

It is well known that TgAbs may interfere in Tg assay and the percentage of patients with circulating TgAbs in DCT varies between 2 to 15% in literature (14, 25, 26); in our series, to our knowledge the largest reported in literature on serum TgAbs incidence in patients with DTC, TgAbs were detectable in 9.7% of cases.

In most previous studies on the usefulness of the Tg assay in the follow-up of DCT patients, cases with circulating TgAbs were excluded. However, it is also known that in these patients we can obtain both under and overestimated Tg values using RIA methods but invariably we obtain underestimated Tg values using IRMA methods (16, 18, 19, 22). Accordingly, the present data show that serum Tg levels measured by an IRMA method, may be elevated in patients with metastases, particularly distant metastases, and circulating TgAbs, even if less frequently than in patients with metastases but without TgAbs. Moreover, it appears of interest to point out that after hormonal withdrawal the sensitivity of Tg assay as tumoral marker is increased, not only in cases without circulating TgAbs as previously reported in literature (11, 12), but also in cases with circulating TgAbs as shown in the present study. This observation may be explained by the increased synthesis and release of Tg under TSH stimulation. So, our data, obtained from a much larger series of patients, strictly confirm the observations suggested in a previous study by Feldt-Rasmussen et al. (17), carried out on a very much smaller group of patients, 72 cases, who reported the finding of high Tg serum levels in some patients with metastatic DTC and circulating TgAbs.

One of the main purposes of this study was to evaluate the possible clinical role of circulating TgAb levels in patients with DCT. Our data show that preoperative TgAb serum levels are not prognosti-

cally useful. On the other hand, after thyroid ablation, the highest TgAb levels were found in patients with metastases, while in most of the patients considered tumor-free circulating TgAbs disappeared or remained at levels lower than the preoperative ones. Our data are in keeping with those recently reported by Pacini et al. (14). Some doubts remain as to why unchanged TbAb serum levels persist in some cases without evidence of metastases. It is possible that microfoci of metastatic tissue, not shown by the currently available diagnostic techniques, may produce Tg and provide the immune system with a continuous supply of antigen. Tg production in these cases may be not detectable just because of circulating TgAbs. If so, these cases should be considered at risk.

In conclusion, this study shows that: i) The prevalence of circulating TgAbs in a large series of patients with DTC is about 10%; ii) Despite the presence of circulating TgAbs, serum Tg may be elevated in some patients with metastases, particularly distant metastases, maintaining in these cases its value as a neoplastic marker; On the other hand a negative value of serum IRMA-Tg is not a meaningful assay, since presence of metastases cannot be excluded; iii) High serum TgAb levels in patients treated for DTC, even in absence of detectable Tg serum levels, may lead one to suspect the presence of neoplastic thyroid tissue.

REFERENCES

1. Van Herle A.J., Uller R.P.
Elevated serum thyroglobulin: a marker of metastases in differentiated thyroid carcinoma.
J. Clin. Invest. *56*: 272, 1975.
2. Lo Gerfo P., Stillman T., Colacchio D., Feind C.
Serum thyroglobulin and recurrent thyroid cancer.
Lancet *1*: 881, 1977.
3. Schossberg A.H., Jacobson J.C., Ibbertson H.K.
Serum thyroglobulin in the diagnosis and management of thyroid carcinoma.
Clin. Endocrinol. (Oxf.) *10*: 17, 1979.
4. Charles M.A., Dodson L.E., Waldeck N., Hofeldt F., Ghaed N., Telepak R., Ownbey J., Burstein P.
Serum thyroglobulin levels predict total body iodine scan findings in patients with treated well-differentiated thyroid carcinoma.
Am. J. Med. *69*: 401, 1980.
5. Ashcraft M.N., Van Herle A.J.
The comparative value of serum thyroglobulin measurements and iodine 131 total body scans in the follow-up study of patients with treated differentiated thyroid cancer.
Am. J. Med. *71*: 806, 1981.
6. Black E.G., Cassoni A., Gimlette T., Harmer C.L., Maisey M.N., Oates G.D.
Serum thyroglobulin in thyroid cancer.
Lancet *2*: 443, 1981.
7. Schlumberger M., Fragu P., Parmentier C., Tubiana M.
Thyroglobulin assay in the follow-up patients with or without normal residual tissue.
Acta Endocrinol. (Copenh.) *98*: 215, 1981.
8. Schneider A.B., Bruce R.L., Goldman J.M., Robbins J.
Sequential serum thyroglobulin determinations, 131 I scans, and 131 I uptakes after triiodothyronine withdrawal in patients with thyroid cancer.
J. Clin. Endocrinol. Metab. *53*: 1199, 1981.
9. Charles M.A.
Comparison of serum thyroglobulin with iodine scans in thyroid cancer.
J. Endocrinol. Invest. *5*: 267, 1982.
10. Girelli M.E., Busnardo B., Amerio R., Scotton G., Casara D., Betterle C., Piccolo M., Pelizzo M.R.
Serum thyroglobulin levels in patients with welldifferentiated thyroid cancer during suppressive therapy: study on 429 patients.
Eur. J. Nucl. Med. *10*: 252, 1985.
11. Girelli M.E., Piccolo M., Nacamulli D., Casara D., Amerio R., Busnardo B.
Relationships between radioiodine uptake and thyroglobulin production by nodal versus distant metastases of thyroid cancer.
In: Jaffiol C., Milhand G. (Eds.), Thyroid cancer.
Elsevier Science Publishers B.V. 387, 1985.
12. Girelli M.E., Busnardo B., Amerio R., Casara D., Betterle C., Piccolo M.
Critical evaluation of serum thyroglobulin (Tg) levels during thyroid hormone suppression therapy versus Tg levels after hormone withdrawal and total body scan: results in 291 patients with thyroid cancer.
Eur. J. Nucl. Med. *11*: 333, 1986.
13. Black E.G., Sheppard M.C., Hoffenberg R.
Serial serum thyroglobulin measurements in the management of differentiated thyroid carcinoma.
Clin. Endocrinol. (Oxf) *27*: 115, 1987.
14. Pacini N., Mariotti S., Formica N., Elisei R., Anelli S., Capotorti E., Pinchera A.
Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumor outcome.
Acta Endocrinol. (Copenh.) *119*: 373, 1988.

15. Muller-Gartner H.W. Schneider C.
Clinical evaluation of tumor characteristics predisposing serum thyroglobulin to be undetectable in patients with differentiated thyroid cancer.
Cancer *61*: 976, 1988.

16. Mariotti S., Cupini C., Giani C., Lari R., Rolleri E., Falco A., Marchisio M., Pinchera A.
Evaluation of a solid-phase immunoradiometric assay (IRMA) for serum thyroglobulin: effect of antithyroglobulin autoantibodies.
Clin. Chim. Acta *123*: 347, 1982.

17. Feldt-Rasmussen U., Holten I., Hansen H.S.
Influence of thyroid substitution therapy and thyroid autoantibodies on the value of serum thyroglobulin in recurring thyroid cancer.
Cancer *51*: 2240, 1983.

18. Feldt-Rasmussen U., Schlumberger M.
European interlaboratory comparison of serum thyroglobulin measurement.
J. Endocrinol. Invest. *11*: 175, 1988.

19. Feldt-Rasmussen U., Krogh-Rasmussen A.
Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb *in vitro* and *in vivo*.
J. Endocrinol. Invest. *8*: 571, 1985.

20. Krogh-Rasmussen A., Feldt Rasmussen U., Date J., Witten J., Hansen H.S., Fungh-Rosemberg H.
Comparison of two assays for serum thyroglobulin and relation to thyroglobulin autoantibodies.
In: C. Jaffiol, Milhaud (Eds.), Thyroid cancer.
Excerpta Medica, Amsterdam, 1985, p. 379.

21. Van Herle A.J., Uller R.P., Matthews N.L., Brown J.
Radioimmunoassay for measurement of thyroglobulin in human serum.
J. Clin. Invest. *52*: 1320, 1973.

22. Schneider A.B., Pervos R.
Radioimmunoassay measurement of human thyroglobulin. Effect of anti-thyroglobulin autoantibodies.
J. Clin. Endocrinol. Metab. *47*: 126, 1978.

23. Preissner C.M., Klee G.G., Krco C.J.
Nonisotopic "sandwich" immunoassay of thyroglobulin in serum by the biotin-streptavidin technique: evaluation and comparison with an immunoradiometric assay.
Clin. Chem. *34*: 1794, 1988.

24. Busnardo B., Vangelista R., Girelli M.E., Bui F., Lazzi C.
TSH levels and TSH response to TRH as a guide to the replacement treatment of patients with thyroid carcinoma.
J. Clin. Endocrinol. Metab *42*: 901, 1976.

25. Amino N., Pysher T., Cohen E., DeGroot L.J.
Immunological aspects of human thyroid cancer.
Cancer *36*: 963, 1975.

26. De Groot L.J., Hoye K., Refetoff S., Van Herle A.J., Asteris G.T., Rochman H.
Serum antigen and antibodies in the diagnosis of thyroid cancer.
J. Clin. Endocrinol. Metab. *45*: 1220, 1976.

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ACTA ENDOCRINOLOGICA, (1985) Vol. 108, pp. 151

Eur J Nucl Med, (1981). Vol. 6, No. 11, pp. 515-520

ENDOCRINOL SUPPL. (1985) 108 (267), 151

J SAITAMA MED SCH, (1989) 16 (3), 353-364

CANCER RESEARCH, (1975 Oct) 35 (10) 2689-92

KLINISCHE WOCHENSCHRIFT, (1982 May 3) 60 (9) 457-64

CHINESE MEDICAL JOURNAL, (1989 Apr) 102 (4) 282-9

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION, (1990 Oct) 13 (9) 737-42

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MEASUREMENT AND CLINICAL SIGNIFICANCE OF THYROID MICROSOMAL AND THYROGLOBULIN ANTIBODIES BY ENZYME-LINKED IMMUNOSORBENT ASSAY *

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Using the thyroid microsomal antigen(TMAg) prepared by affinity chromatographygel filtration method of sufficient purity, we measured the TM antibody (TMAb) level by an enzyme-linked immunosorbent assay (ELISA) in 103 normal persons and 183 patients with various thyroid disorders (Hashimoto's thyroiditis, Graves' disease, hypothyroidism, subacute thyroiditis, thyroid cancer, thyroid adenoma and simple goiter). The thyroglobulin antibody (TCAb).T3 and T4 were also measured at the same time. Based on the measurement of TMAb and TGAb of the thyroid diseases and analysis of their incidences and titer, our data strongly support that ELISA using purified TM and TG is a very useful and promising method for diagnosis and distinguishing autoimmune from non-autoimmune thyroid disease, and also can be employed in monitoring the development and studying the pathogenesis of the disease. We found that there is a negative correlation between TMAb titer and T3, T4 values ($P<0.01$) which has not been reported before in the literature. According to the result of the study, we suggest an immunological classification of thyroid diseases.

Several antigen-antibody (Ag-Ab) systems are found in sera from patients with thyroid diseases. Among these thyroglobulin (TG) antibody was first revealed in Hashimoto's thyroiditis and another distinct Ag-Ab system, the thyroid microsomal (TM) Ag-Ab system has also been described[1]. Although the TMAg-Ab plays the same role as TGAg-Ab, the study of TMAg-Ab, compared with that of TGAg-Ab, lagged much behind mainly due to the lack of purified TM preparation. It is well known that most TM preparations were heavily contaminated with TG, and false positive results for TMAb are unavoidable. Thus one must take account of the TG contamination in any assay for autoantibodies against the TMAg. Here we submit an enzyme linked immunosorbent assay (ELISA) using a purified TMAg. TGAb, T3 and T4 were also measured. Some interesting findings with clinical significance are reported.

MATERIAL AND METHODS

Preparation of TMAg. The procedures for the TMAg preparation was described in detail elsewhere[2]. Briefly speaking, we have developed an affinity chromatographygel filtration (AC-GF) device in which an affinity chromatography column is connected with a Sepharose 4B filtration column. Sepharose G50 is filled in between the rubber stopper and Sepharose 4B in the GF column. The crude TM prepared by conventional ultracentrifugation is applied to the TGAb coupled AC column first through which the contaminated TG is removed, the eluate passing directly into the GF is further

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chromatographed. Fractions with peak antigenic activity were used as TMAg.

Preparation of TGAg. TGAg was prepared by Sephadex G200 chromatography and DEAE–C22 ion exchange chromatography[3].

Serum samples. A total of 103 normal serum samples were obtained from healthy blood donors (56 women, 47 men) varying in age from 19 to 49, and 83 patients' sera from a variety of thyroid disorders varying in age from 19 to 70. They were diagnosed on the basis of clinical manifestation, detection of thyroid autoantibodies and thyroid function (Table 1). Patients of groups 1–4 were under treatment, while serum samples in groups 5 and 6 were obtained before operation. All serum samples were kept at −60 C.

Table 1. Incidences of TMAb and TGAb in thyroid diseases

Group	Disorders	Number(F,M)*	Positive cases (%)		Diagnosis method
			TMAb	TGAb	
1	Hashimoto's thyroiditis	41 (36 , 5)	38 (92.7)[a]	36 (87.8)[e]	
2	Graves' diseasr	54 (43 , 11)	49 (90.7)[a]	33 (61.1)[f]	T3 or / and T4 > normal
3	Hypothyroidism	23 (19 , 4)	19 (82.6)[b]	18 (78.3)[g]	T3 or / and T4 < normal
4	Subacute thyroiditis	13 (11 , 2)	6 (46.2)[c]	5 (38.5)[h]	
5	Thyroid cancer	26 (22 , 4)	10 (38.5)[d]	5 (19.1)[i]	Pathology+
6	Thyroid adenoma	16 (14 , 2)	2 (12.5)	1 (6.3)	Pathology+
7	Simple goiter	10 (6 , 4)	1 (10.0)	0 (0)	
8	Normal	103 (56 , 47)	5 (4.9)	3 (2.9)	

*F = Female , M = Male

a . P < 0.01 , compared with Groups 4–8.

b . P < 0.05 , compared with Group 4 ; P < 0.01 , compared with groups 5–8.

c . P < 0.05 , compared with Group 6 ; P < 0.01 , compared with Group 8.

d . P < 0.01 , compared with Group 8.

e . P < 0.01 , copmared with Groups 2 , 4–8.

F . P < 0.01 , compared with groups 5–8.

g . P < 0.05 , compared with Group 4 ; P < 0.01 , compared with groups 5–8.

h . P < 0.01 , compared with Groups 6 and 7 ; P < 0.01 , compared with Group 8.

i . P < 0.01 , compwred with Group 8.

ELISA for TMAb. To each well of microplate 200 μl of TMAg solution (60 μg / ml, pH 9.6, 0.05 M carbonate buffer solution) was added. The plate was first incu-

bated at 37 C for 2 hours, and then 4 C overnight. The coating fluid was flicked off next day, the plate was washed with pH 7.4, 0.02 M PBS–Tween 20 three times, each of 3 minutes. The serum sample was diluted to 1:100 with washing fluid, 200 μl diluted serum was added to the coated well in duplicates. After incubating at 37 C for one hour, the plate was washed for three times each of 3 minutes. Enzyme–linked rabbit–anti–human IgG (Lanzhou Institute of Biologic Products, Lot No.85002) was diluted with washing fluid to 1:100, and 200 μl / well was added to the plate. After incubating at 37 C for one hour, the plate was again washed 3 minutes for three times. To each well 200 μl o–phenylenediamine (OPD) solution (40 mg OPD, pH 5.0, 0.1 M citrate phosphate buffer 100 ml, 30% H_2O_2 0.15 ml) was added. The plate was kept at 37C for 10 minutes, then 2N H_2SO_4 was added to terminate the reaction. The A values were measured at 403 nm using an ELISA reader (MR 580, Beckman, USA). The titer of serum antibodies is expressed as A value. Negative and positive control sera were included in each test.

ELISA for TGAb. The procedure was the same as that for TMAb except that TGAg (20 μg / ml) was used to coat the microplate.

Radioimmunoassay of T3 and T4. T3 and T4 were measured by radioimmunoassay (RIA) kit (Isotope Laboratory, Tianjin Medical College, Tianjin) according to the instructions.

RESULTS

Normal values of TMAb and TGAb. The normal values (A values) of TMAb and TGAb in 103 normal Chinese adults are 0.07 and 0.06 respectively, and the respective incidences are 4.9% (5 / 103) and 2.9% (3 / 103).

Incidences of TMAb and TGAb and TGAb in serum samples of patients with thyroid diseases. The incidences of TMAb and TGAb in serum samples of patients with thyroid diseases are shown in Table 1. For the convenience of analysis, the positive degree of TMAb and TGAb is arbitrarily graded as following: +, A value 0.07–0.20 (for TGAb, 0.06–0.20); ++, 0.21–0.30; +++, over 0.30. The relationship between mean A value and incidence of autoantibodies in various thyroid diseases is shown in Table 2. Although there is a rather high incidence of TMAb in Graves' hyperthyroidism patients, the positivity is low being 4% and 3% +++ for TMAb and TGAb respectively. However, Hashimoto's thyroiditis patients are the first among various thyroid diseases with regard to incidence and degree of positivity of TMAb and TGAb, showing 39.4% and 47.2%+++ respectively, almost 10–15 times higher than those seen in Graves' disease.

Statistical analysis. Table 1 shows the significant differences of TMAb and TGAb levels among various groups.

We have calculated the coefficient of correlation between TMAb, TGAb and T3, T4. From Table 3 we can see an interesting result, in Hashimoto's thyroiditis, namely, a significant negative correlation between TMAb and T3, T4 and between TGAb and T4, but not in other pairs, which has not been mentioned in the literature.

Comparisons were made between the TGAb and TMAb from different authors by different methods (Table 4). The incidence obtained by measurement with ELISA in Graves' hyperthyroidism patients is higher than

Table 2. The inicidence and positivity of TMAb and TGAb in thyroid diseases

Disease		+		++		+++		Total	
		No.	%	No.	%	No.	%	No.	%
Hashimoto's thyroiditis	TMAb	7	18.4	16	42.2	15	39.4	38	100.0
	TAGb	11	30.6	8	22.2	17	47.2	36	1000.0
Graves' disease	TMAb	16	32.7	31	63.3	2	4.0	49	100.0
	TGAb	20	60.6	12	36.4	1	3.0	33	100.0
Hypothyroidism	TMAb	6	31.6	11	57.9	2	10.5	19	100.0
	TGAb	5	27.8	6	33.3	7	38.9	18	100.0
Subacute thyroiditis	TMAb	5	83.3	1	16.7	0	0	6	100.0
	TGAb	3	60.6	2	40.0	0	0	5	100.0
Thyroid cancer	TMAb	10	100.0	0	0	0	0	10	100.0
	TGAb	5	100.0	0	0	0	0	5	100.0
Thyroid adenoma	TMAb	2	100.0	0	0	0	0	2	100.0
	TGAb	1	100.0	0	0	0	0	1	100.0
Simple goiter	TMAb	1	100.0	0	0	0	0	1	100.0
	TGAb	0	0	0	0	0	0	0	0

Table 3. Coefficient of correlation between TMAb, TGAb and T3, T4

Match pair		Hashimoto's thyroiditis	Graves' disease	Hypothyroidism	Subacute thyroiditis
TMAb	T3	−0.3955[a]	0.1424	−0.0532	−0.2322
	T4	−0.3955[a]	0.1543	−0.1045	−0.1700
TGAb	T3	−0.2442	−0.0092	−0.1669	−0.0482
	T4	−0.2748[b]	−0.0868	−0.2099	0.1305

a. $P<0.01$; b. $P<0.05$

those botained with indirect hemagglutination (IHA) and RIA, while the difference in Hashimoto's thyroiditis is not significant.

DISCUSSION

It is well known that TMAb and TGAb play an important role in the pathogenesis of some thyroid diseases, and with salient feature. The conventional ultracentrifugation method of crude TMAg preparation has some disadvantages.[11,12] The contamination of TG affects greatly the incidence measured and the objectivity of investigation. This trouble may be overcome by removing most contaminated TG from the crude ultracentrifuged preparation with the AC–GF device, and establishing a sensitive ELISA with the purified TMAg, with these measures the measurement is more precise.

From Table 4 it can be seen that the antibody incidence in Graves' hyperthyroidism measured by ELISA is greatly increased. Graves' hyperthyroidism belongs to the low positivity group. Antibody can be detected by a method of higher sensitivity.

Table 4. Comparison of incidences of TMAb and TGAb measured by different methods

	TMAb (%)		TGAb (%)		References
	ELISA[a]	Non-ELISA	ELISA[a]	Non-ELISA	
Graves' disease	90.7		61.1		
		71.7 (IHA)[b]		48.9 (IHA)[b]	4
		86.0 (IHA)		29.0 (IHA)[c]	5
		75.0 (RIA)[b]			6
		36.3 (RIA)[c]			7
Hasbimoto's thyroiditis	92.7		87.7		
		96.0 (IHA)		81.1 (IHA)	4
		95.0 (IHA)		59.0 (IHA)[b]	5
		94.1 (RIA)			6
		90.3 (RIA)			7
		20.0 (RIA)[c]			8
Normal	4.9		2.9		
		7.0 (IHA)		3.4 (IHA)	9
		6.0 (IHA)		4.0 (IHA)	10
		3.1 (RIA)			6
		5.0 (RIA)			7

a. Values measured in this laboratory;

b. $P < 0.05$, compared with that measured by ELISA in this laboratory;

c. $P < 0.01$, ibid.

So the differences in incidences of autoantibodies may reflect different sensitivities of the method. ELISA has the advantages of sensitivity, specificity, simplicity, reproducibility and objectivity and is good for detecting and measuring TMAb and TGAb.

The TMAb and TGAb levels in Graves' hyperthyroidism, Hashimoto's thyroiditisand hypothyroid are significantly higher than those in subacute thyroiditis, thyroid cancer, thyroid adenoma and simple goiter. The mean titer and incidence of both autoantibodies in Hashimoto's thyroiditis, a classic autoimmune disease, rank first among the various thyroid disorders, and the positivity is rather high having 39.4% and 47.2% graded as +++ for TMAb and TGAb respectively (Table 2).

In patients with Hashimoto's thyroiditis, we found a significant negative correlation between TMAb level and T3, T4, and also a significant negative correlation between TGAb level and T4 (Table 3). This observation suggests that the thyroid function in Hashimoto's thyroiditis is closely related with autoimmune pathogenetic mechanisms. Primary tissue injury due to some unknown causes releases TMAg into the circulation which inducesautoantibody (TMAb) production, thus triggering a series of events including complement–mediated cytotoxicity[13] and antibody–dependent K cell–mediated cytotoxicity (ADCC)[14].

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Based on the experimental data and our analysis, we postulate a pathogeneticscheme of TMAb in Hashimoto's thyroiditis (Fig 1). Primary tissue injury due to some unknown cause releases TMAg into circulating which induces autoantibody(TMAb) production, thus triggering a series of events observed clinically, and in the laboratory examination. The TMAb or the complex formed with corresponding antigen will bring about K cell–mediated or complement–mediated cytotoxicity. As the tissue being further damaged, the destruction of microsome is succeeded by reduction of protein–bound iodine[15] and decrease of T3 and T4 synthesis.[16] Moreover, the combination of TMAb and tissue will affect the binding of TSH with thyroid cell, also resulting reduction of T3 and T4. These bring about what we reveal in this study, a significant negative correlation between increase of TMAb and decrease of T3 and T4.

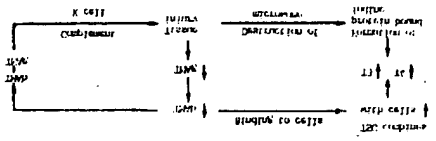

Fig 1. The relationship between TMAb and thyroid function in Hashimoto's thyroiditis

The incidence of TMAb is evidently higher than that of TGAb in Graves' disease, therefore, the former has more important diagnostic value for this disease. Another characteristic is that only 4% and 3% of these two types of autoantibodies were graded as +++ (Table 2), almost more than 10 times lower than those found in Hashimoto's thyroiditis. Among these patients there may be some cases complicated with local Hashimoto's thyroiditis.[17] There is no positive correlation between antibody level and T3, T4 in Graves disease. For many years, the pathogenetic mechanisms have been investigated from various aspects, but so far no conclusive result has been obtained. Evidently, high levels of TMAb and TGAb suggest that autoimmune mechanism plays an important role too, but the mechanism is different from that of Hashimoto's thyroiditis. Whether TMAb has an effect similar to that of long acting thyroid stimulator (LATS) has not been studied yet. Such investigation would be very interesting and tempting.

The level of TMAb and TGAb of hypothyroidism patients is next to that of Hashimoto's thyroiditis patients. The reduction of thyroid function of most patients is often secondary to thyroiditis, and such patients are often clinically atypical. In a study of the TMAb effect in the treatment of patients, it was found that TMAb may reduce the sensitivity of the thyroid to TSH leading to an enhanced secretion of TSH and promote the development of hypothyroidism[16]. If one takes into account the antibody level and thyroid function together with the case history, a definite diagnosis could be made for those ascribed for a long time to hypothyroidism.

The incidence of TMAb in subacute thyroiditis is significantly lower than in Hashimoto's disease. The positivity shows only + to ++, and there is no correlation between antibody level and T3, T4. These findings support that it is a nonautoimmune disease. As the exact pathogenesis of subacute thyroiditis is not elucidated the mechanism of the increase of antibody titer in some patients need further study.

Both TMAb and TGAb levels in thyroid adenoma and simple goiter approximate to

those of the normal adults. These diseases belong to non–autoimmune thyroid diseases. It is of interest to note effect of the higher antibody but lower positivity of the incidence in thyroid cancer patients than normals($P < 0.01$) for TMAb and TGAb.This may be caused by the release of thyroid antigen and prevented by the immune status of the cancer patient. The relationship of antibody production and types of thyroid cancer is under study at present.

On the basis of our experimental results, thyroid diseases may immunologically be classified into high autoantibody level group (autoimmue thyroid disease) and low autoantibody level group (non–autoimmune thyroid disease). The formar may further be divided into strong positivity subgroup (Hashimoto's thyroiditis and hypothyroidism) and non–strong positivity subgroup (Graves' hyperthyroidism). The low autoantibody group or non–autoimmune thyroid disease group may be subdivided into high incidence subgroup (subacute thyroiditis and thyroid cancer) and low incidence subgroup (thyroid adenoma and simple goiter). This immunological classification of thyroid diseases is useful to basic and clinical investigations.

REFERENCES

1. Wang L, Zheng WF. Thyroid microsomal antigen–antibody system and its clinical significance. Chin J Immunol 1987;3:29.

2. Wang L, Zheng WF. An affinity chromatography–gel filtration device for preparing thyroid microsomal antigen. J Immunol Methods 1987;102:221.

3. Lu FX, et al. Purification of thyroglobulin antigen. J Tianjin Med Coll 1979;2:97.

4. Dai ES, et al. Measurement of thyroid microsomal antibody by hemagglutination method and its values in the diagnosis of autoimmune thyroiditis. Shanghai Medicine 1981;4:6.

5. Amino N, et al. Measurement of circulating thyroid microsomal antibodies by the tanned red cell hemagglutination technique: Its usefulness in the diagnosis of autoimmune thyroid diseases. Clin Endocrinol 1976;5:115.

6. Mariotti S, et al. Comparison of radioassay and hemagglutination methods for anti–thyroid microsomal antibodies. Clin Exp Immunol 1978;34:118.

7. Xiao XX, et al. Clinical application of radioimmunoassay in detecting serum thyroid microsomal antibody. Shanghai Immunol J 1985;5:45.

8. Kung VT, et al. Double–antibody radioimmunoassay of thyroid microsomal antibody in serum. Clin Chem 1981;27:39.

9. Bjoro T, et al. Thyroid antibodies in blood donors: prevalence and clinical significance. Acta Endocrinol 1984;105:324.

10. Cayzer I, et al. An evaluation of the two new hemagglutination test or the rapid diagnosis of autoimmune thyroid diseases. J Clin Pathol 1978;31:1147.

11. Weetman AP, et al. Enzyme–linked immunoassay of monoclonal and serum microsomal autoantibodies. Clin Chim Acta 1983;138:237.

12. Mariotti S, et al. A new solid phase immunoradiometric assay for antithyroid microsomal antibody. J Clin Endocrinol Metab 1983;56:467.

13. Parkes AB, et al. The distribution of microsomal and thyroglobulin antibody activity among the IgG subclasses. Clin Exp Immunol 1984;57:239.

14. Bogner U, et al. Antibody–dependent cell mediated cytotoxicity against human thyroid cells in Hashimoto's throiditis but not Graves' disease. J Chin Endocrinol Metab 1984;59:734.

15. Nakagawa H, et al. Stimulation of protein–bound iodine formation by lipid extracts from hog thyroid microsomes. Endocrinol Jpn 1981;28:409.

16. Foldes J, et al. Reduced sensitivity of the

thyroid to thyrotrophic hormone and LATS following treatment with antimicrosomal antibodies. Acta Endocrinol 1973;74:675.

17. Bai Y, et al. Studies on diagnosis of autoimmune thyroiditis. Acta Chin Acad Med Sci 1984;6:179.

BEIJING GALL AND KIDNEY STONE MEDICAL CENTER FOUNDED

The Beijing Gall and Kidney Stone Medical Center, the first of its kind in China, was founded on March 8, 1989.

The center is jointly founded by the Urological Institute of Beijing Medical University, the Shantou–Yat Chau Medical Instrument Company Ltd. and the Yat Chau Company Ltd. of Hong Kong.

According to information given at the founding ceremony, the Urological Institute of Beijing Medical University took the lead in the research. In the past two years, it treated more than 3 600 patients suffering from kidney stones and 50 cases of gallstones. Ninety–nine per cent of the kidney stone patients were cured as well as 95 per cent of the gallstone sufferers.

The Dputy–Director of the Institute, Professor Guo Ying–lu, said there are now about 10 factories producing equipment for crashing kidney or gall stones in China. But the kind of machine guided by B–type ultrasonic diagnostic apparatus was first turned out by the Shantou–Yatchau Medical Instrument Company Ltd. The machine freed the patients from the pain of surgical operation, the fear of anesthesia and the harm of X–rays.

The machine has been used ot treat 10 000 patients with a success rate of 100 per cent.

ENGLISH–CHINESE PRACTICAL GUIDE TO TRADITIONAL CHINESE MEDICINE TO BE PUBLISHED

China's first practical English–Chinese Guide to Traditional Chinese Medicine will be off press in April 1989.

The 12–volume guide, published by the Shanghai Traditional Chinese Medicine College, covers all aspects of traditional Chinese medicine including Qigong, acupuncture and moxibustion.

Two of the volumes deal with the operation of a traditional Chinese medicine clinic. Two volumes are devoted to basic theory.

The books are written, translated and edited by experts from the State Administration of Traditional Chinese Medicine and Pharmacology, the Chinese Academy of Traditional Chinese Medicine, the Shanghai Traditional Chinese Medicine College, Shanghai Medical University, Shandong University and several other colleges.

A number of foreign medical experts also participated in editing.

Special attention has been paid to the practical use of the traditional Chinese medicine. Both the Chinese and English versions are concise and are accompanied with illustrations and color photos.

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ACTA ENDOCRINOLOGICA, (1985) Vol. 108, pp. 151

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ENDOCRINOL SUPPL. (1985) 108 (267), 151

J SAITAMA MED SCH, (1989) 16 (3), 353-364

CANCER RESEARCH, (1975 Oct) 35 (10) 2689-92

KLINISCHE WOCHENSCHRIFT, (1982 May 3) 60 (9) 457-64

CHINESE MEDICAL JOURNAL, (1989 Apr) 102 (4) 282-9

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION, (1990 Oct) 13 (9) 737-42

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Thanks,

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Klinische Wochenschrift

Serum-Thyreoglobulinspiegel als Tumormarker bei Schilddrüsencarcinom

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Significance of Thyroglobulin as a Tumor Marker in the Serum of Patients with Differentiated Thyroid Carcinoma: Longitudinal and Cross-Sectional Studies

Summary. For evaluating the clinical significance of thyroglobulin measurements for the follow-up of patients with differentiated thyroid carcinoma, thyroglobulin was determined radioimmunologically during the past 2 years (up to 12 times) in 40 patients after withdrawal of thyroid hormone. Thyroglobulin values were compared with whole-body scintigrams after radioiodine. Thyroglobulin antibodies, which may interfere in the radioimmunoassay for thyroglobulin, were also estimated by a radioimmunologic method.

In the majority of cases, thyroglobulin levels corresponded to the scintigrams, however, the thyroglobulin level appeared to be a more precise index for changes in tumor tissue mass. In one patient the scintigram was negative, whereas considerable amounts of thyroglobulin were measured in the serum: X-ray tomography revealed a lung metastase in this case. On the other hand, thyroglobulin was undetectable in the sera of patients who exhibited distinct metastases in the scintigram.

Thyroglobulin can be regarded as a tumor marker in patients thyroidectomized for differentiated thyroid carcinoma. However, its determination can certainly not replace whole-body scintigraphy as postulated by several authors, although thyroglobulin measurement appears to be superior to scanning in some cases. A combined application of iodine scanning and thyroglobulin radioimmunoassay is thus advisable in the follow-up of patients with differentiated thyroid carcinoma.

Key words: Thyroglobulin – Thyroid carcinoma – Radioiodine therapy – Thyroglobulin antibodies – Metastases

Sonderdruckanfragen an: Prof. Dr. H. Schatz (Adresse s. nach Literatur)

Zusammenfassung. Um festzustellen, welche Wertigkeit der Thyreoglobulinmessung im Serum wegen differenzierten Schilddrüsencarcinoms thyreoidektomierter Patienten zukommt, wurde während der letzten 2 Jahre prospektiv der Verlauf der Thyreoglobulinspiegel (bis zu zwölfmal) bei 40 Patienten mit follikulärem oder papillärem Schilddrüsencarcinom nach Absetzen der Schilddrüsenhormongabe radioimmunologisch bestimmt und mit den Radiojodszintigrammen verglichen. In jeder Serumprobe wurden auch die Thyreoglobulinantikörper, ein möglicher Störfaktor der Thyreoglobulinbestimmung, radioimmunologisch gemessen.

In der Mehrzahl der Fälle entsprachen Thyreoglobulinspiegel und Szintigramm einander, der Thyreoglobulinwert erlaubte aber eine exaktere Quantifizierung der Veränderungen der Tumormasse als die optische Beurteilung der Szintigramme. Bei einem der Patienten ergab die Szintigraphie einen völlig negativen, die Thyreoglobulinmessung hingegen einen deutlich positiven Befund: Bei diesem Patienten deckte die Röntgentomographie eine Lungenmetastase auf. Umgekehrt stellten sich bei Patienten ohne nachweisbares Thyreoglobulin radiojodspeichernde Metastasen im Szintigramm dar.

Thyreoglobulin kann bei wegen differenzierten Schilddrüsencarcinoms thyreoidektomierten Patienten als Tumormarker betrachtet werden. Die Thyreoglobulinbestimmung kann jedoch nicht, wie es von einigen Autoren postuliert wurde, die Ganzkörperszintigraphie ersetzen, obwohl sie sich der Szintigraphie in manchen Fällen als überlegen erweist. Es empfiehlt sich daher die Kombination der Szintigraphie mit der Thyreoglobulinmessung für die Verlaufskontrolle von Patienten mit differenziertem Schilddrüsencarcinom.

Schlüsselwörter: Thyreoglobulin – Schilddrüsencarcinom – Radiojodtherapie – Thyreoglobulinantikörper – Metastasierung

Thyreoglobulin stellt nicht nur die Speicherform für Schilddrüsenhormone. sondern auch ein physiologisches Sekretionsprodukt der Schilddrüse dar (Übersicht [18]), welches sich im zirkulierenden Blut des Menschen radioimmunologisch nachweisen läßt [8, 18]. Im Unterschied zum undifferenzierten Carcinom und zum C-Zellcarcinom der Schilddrüse wird Thyreoglobulin auch von follikulärem und papillärem Schilddrüsenkrebsgewebe gebildet, ist aber klinisch als Tumormarker zunächst nicht brauchbar, da erhöhte Thyreoglobulinspiegel nicht nur bei Patienten mit differenziertem Schilddrüsencarcinom, sondern auch bei anderen Erkrankungen der Schilddrüse wie z.B. der Hyperthyreose oder auch der blanden Struma zu finden sind (Übersicht [18]). Wird das normale Schilddrüsengewebe jedoch zu Beginn der Carcinombehandlung durch totale Thyreoidektomie mit nachfolgender Radiojodgabe eliminiert, so sollte im Serum kein Thyreoglobulin mehr nachweisbar sein, es sei denn, Thyreoglobulin entstammt den differenzierten Zellen von Tumorrestgewebe bzw. einem Lokalrezidiv oder aus Metastasen. Somit könnte der Messung des Thyreoglobulinspiegels für die Verlaufskontrolle von Patienten nach der Ersttherapie von differenzierten Schilddrüsencarcinomen klinische Bedeutung zukommen [1, 2, 3, 4a, 5. 6. 7, 11, 12, 14, 15, 16].

Im Unterschied zu Querschnittsuntersuchungen lag uns keine Publikation über eine größere Serie von Längsschnittuntersuchungen vor. Daher bestimmten wir während der beiden letzten Jahre bei unseren Patienten mit differenziertem Schilddrüsencarcinom prospektiv den Verlauf der Thyreoglobulinspiegel sowie der Thyreoglobulinantikörper und verglichen die Veränderungen der Thyreoglobulinspiegel mit dem Bild der Ganzkörperszintigramme (vgl. 10a).

Patienten und Methoden

Insgesamt 40 Patienten mit follikulärem ($n=19$), papillärem ($n=14$) oder follikulär-papillärem ($n=7$) Schilddrüsencarcinom wurden untersucht, davon 36 prospektiv im Verlauf jeweils vor und nach einer bzw. mehreren (maximal 6) Radiojodtherapien. Die Radiojodtherapien erfolgten in vierteljährlichem Abstand, wobei nach Schilddrüsenhormon-Desubstitution (4 Wochen vor Therapie Wechsel von Thyroxin auf Trijodthyronin, die letzten 10 Tage ohne jegliches Schilddrüsenhormon) am 1. und 7. Tag je 50 mCi Jod-131 intravenös injiziert wurden. Serum für die Thyreoglobulinmessung wurde vor der ersten Dosis und 10 Tage danach gewonnen. Jede Serumprobe wurde radioimmunologisch auf Thyreoglobulinantikörper geprüft. Am 10. Tag wurde mit der Restradioaktivität der therapeutischen Radiojoddosis ein Ganzkörperszintigramm angefertigt. Bei 4 der 40 Patienten war die Radiojodtherapie bereits früher beendet worden und die Szintigramme waren – nach Desubstitution wie oben angegeben – mit einer diagnostischen Dosis von 2 mCi Jod-131 angefertigt worden.

Zum Vergleich wurde Thyreoglobulin auch bei 56 Normalpersonen, 25 Patienten mit Hyperthyreose, 21 Patienten mit blander Struma, 10 Patienten mit anderen Schilddrüsenerkrankungen und 2 Patienten mit C-Zellcarcinom der Schilddrüse gemessen.

Zur Thyreoglobulinmessung dienten die Reagenzien eines Doppelantikörperradioimmunoassays, die uns von der Firma Henning, Berlin, zur Verfügung gestellt wurden und die seit einiger Zeit auch als Testsatz (Tg-RIA „Henning") kommerziell erhältlich sind. Das Antiserum gegen humanes Thyreoglobulin stammte vom Kaninchen, der zweite Antikörper gegen Kaninchengammaglobulin von der Ziege.

Die Thyreoglobulinstandards wurden in Humanserum angesetzt. Der Intraassay-Variationskoeffizient betrug unter Verwendung eines eigenen Poolserums anfangs 8,2% ($n=28$), später 5,0% ($n=10$), der Interassay-Variationskoeffizient 7,4% ($n=10$). Die untere Empfindlichkeitsgrenze setzten wir bei 5 ng/ml an, obwohl in einem großen Teil der Assays noch deutlich herab bis zu 1 ng/ml unterschieden werden konnte.

Alle Seren wurden auf *Autoantikörper gegen Thyreoglobulin* mit dem Radioimmunoassay von CIS (Isotopendienst West, Dreieich) geprüft. Vergleichsweise wurde auch die Wiederfindung zugesetzten Thyreoglobulins bestimmt.

Die *Szintigraphie* wurde an einem Scanner (Szintimat II, Siemens, Erlangen) mit der Restradioaktivität am 10. Tage nach der ersten therapeutischen Dosis bzw. nach einer diagnostischen Dosis von 2 mCi Jod-131 durchgeführt. Die Wiedergabe der Farbszintigramme erfolgt auf den Abbildungen in schwarz-weiß.

Ergebnisse

Bei 56 schilddrüsengesunden Kontrollpersonen ergab sich ein Mittelwert ± SEM des Serumthyreoglobulinspiegels von 20,2 ± 2,3 ng/ml (Bereich: < 5–79). Knapp

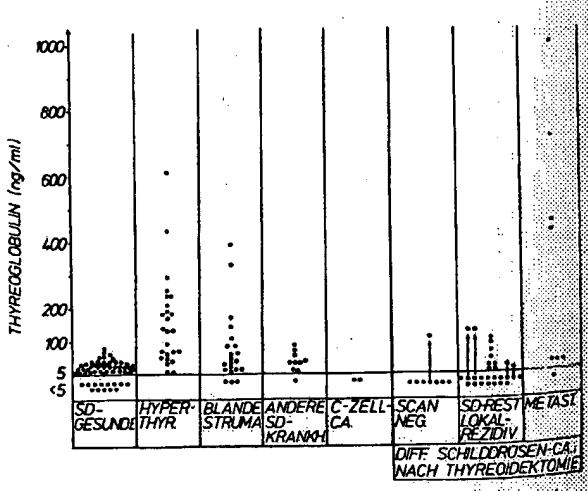

Abb. 1. Thyreoglobulin im Serum von Schilddrüsen(SD)Gesunden ($n=56$), von Patienten mit Hyperthyreose ($n=25$), mit blander Struma ($n=21$) mit anderen Schilddrüsenerkrankungen ($n=10$) und von zwei Patienten mit C-Zellcarcinom der Schilddrüse. Die drei rechten Spalten zeigen die Thyreoglobulinspiegel unmittelbar *vor* Radiojodgabe bei wegen differenzierten Schilddrüsencarcinoms thyreoidektomierten Patienten nach Hormon-Desubstitution (s. auch Text). In den 5 Fällen, bei denen Thyreoglobulin nur 10 Tage *nach* Beginn der Radiojodtherapie nachweisbar war, nicht jedoch vor Radiojodtherapie, ist dieser posttherapeutische Wert zusätzlich als Sternchen eingezeichnet. „Scan neg." = keine Radiojodspeicherung am Hals und keine speichernden Metastasen (in dieser Gruppe befinden sich auch 4 Patienten mit diagnostischem Scan nach Beendigung der Radiojodtherapie, s. Text). „SD-Rest Lokalrezidiv" = Radioaktivitätseinlagerung im Bereich des Schilddrüsenbettes. „Metast." = speichernde Metastasen

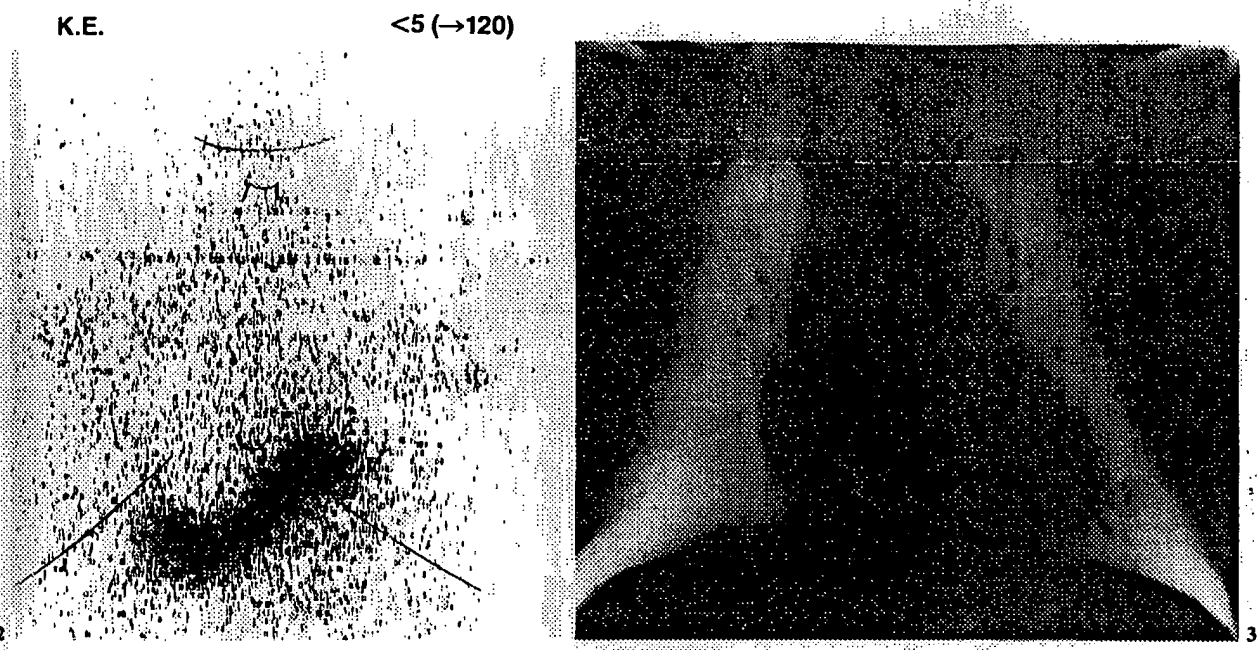

Abb. 2. Keine Radiojodspeicherung über Hals und Stamm nach initialer Radiojodtherapie bei einer Patientin nach totaler Thyreoidektomie wegen differenzierten Schilddrüsencarcinoms. Eingezeichnet sind Operationsschnitt am Hals, Schwertfortsatz und Rippenbogen. Die Radioaktivität im Epigastrium entspricht der Ausscheidung von radioaktivem Jod über die Magenschleimhaut. Der Thyreoglobulinspiegel lag vor Radiojodtherapie unter der sicheren Nachweisgrenze von 5 ng/ml, nach Radiojodtherapie (Wert in Klammern) betrug er jedoch 120 ng/ml. Die Tomographie der Lunge deckte eine Metastase auf (Abb. 3)

Abb. 3. Tomographischer Nachweis einer Lungenmetastase links von der Herzspitze, die nur über die Thyreoglobulinmessung, nicht jedoch durch Radiojodszintigraphie (s. Abb. 2) erfaßbar war

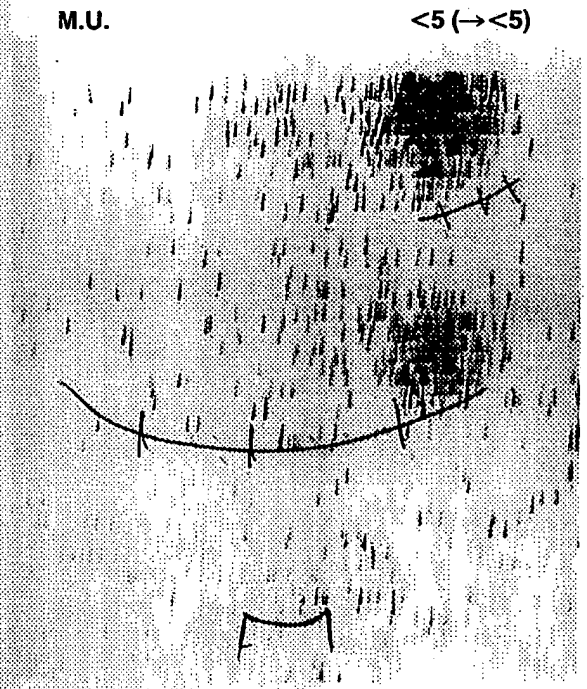

Abb. 4. Szintigraphischer Metastasennachweis bei einer Patientin mit einem Thyreoglobulinspiegel unter 5 ng/ml sowohl vor als auch nach (Wert in Klammern) Radiojodtherapie. Eingezeichnet Jugulum und Operationsschnitte am Hals

ein Viertel der Patienten hatte Werte unter 5 ng/ml. Für hyperthyreote Patienten ($n=25$) errechneten sich $157,5 \pm 27,7$ ng/ml (Bereich: 5–611), für Patienten mit blander Struma ($n=21$) $77,8 \pm 22,9$ ng/ml (Bereich: <5–394) (Abb. 1). Bei den hyperthyreoten Patienten ist zu berücksichtigen, daß sich in einem beträchtlichen Teil der Seren endogene Antikörper gegen Thyreoglobulin fanden, so daß diese Thyreoglobulin-Meßwerte nicht vergleichbar bzw. nicht stets eindeutig interpretierbar sind.

Die Resultate der Querschnittsuntersuchungen bei den thyreoidektomierten Carcinompatienten zeigt Abb. 1. Aus Gründen der Übersichtlichkeit ist auch bei den Patienten, die mehrmals mit Jod-131 behandelt wurden, nur ein Thyreoglobulin-Meßwert, der vor der ersten Radiojodtherapie der Verlaufsserie, bzw. der Thyreoglobulin-Meßwert nach Desubstitution vor dem diagnostischen Scan, als Punkt aufgetragen. Zehn Tage nach Radiojodtherapie fand sich häufig ein – teilweise sehr ausgeprägter – Anstieg des Thyreoglobulinspiegels, der bei den vor Therapie Thyreoglobulin-positiven Patienten im Mittel 26,0% betrug. Diese posttherapeutischen Werte sind lediglich in den 5 Fällen, bei denen Thyreoglobulin nur nach und nicht vor Radiojodtherapie nachweisbar war, zusätzlich als Sternchen aufgetragen.

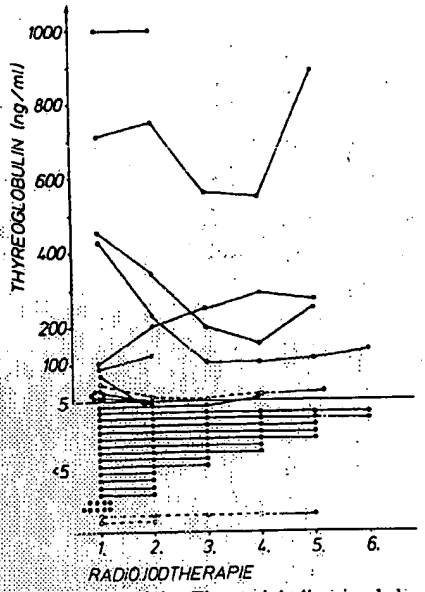

Abb. 5. Verlauf der Thyreoglobulinspiegel, bestimmt jeweils nach Schilddrüsenhormon-Desubstitution vor Radiojodtherapie. Sechs der anfangs Thyreoglobulin-positiven Patienten zeigten einen Abfall, allerdings kam es bei zwei Fällen zu einem deutlichen Wiederanstieg. Von den ursprünglich Thyreoglobulin-negativen Patienten wurde nur bei einem Fall im Verlauf ein Thyreoglobulinanstieg in den meßbaren Bereich gefunden. Nur bei drei der 40 Patienten fanden sich radioimmunologisch mit dem Assay von CIS Thyreoglobulinantikörper (offene Kreise, strichlierte Linien), die bei zwei der Patienten im Verlauf der Beobachtung verschwanden. Nie konnte ein Neuauftreten von Thyreoglobulinantikörpern nachgewiesen werden

1. Gruppe „Scan negativ" (Abb. 1): Bei 4 dieser 7 Fälle war bereits die Radiojodtherapie beendet gewesen. Diese 4 Patienten mit negativem diagnostischen Scan waren auch Thyreoglobulin-negativ. Die anderen 3 Fälle hatten noch eine Radiojodtherapie erhalten: Zweimal war beim letzten vorangegangenen, posttherapeutischen Scan noch radiojodspeicherndes Gewebe nachweisbar gewesen, so daß eine weitere Therapie angesetzt worden war. Beim 3. Patienten handelte es sich um die erste Radiojodtherapie im Anschluß an die (hier tatsächlich „total" erfolgte) Thyreoidektomie. In allen 7 Fällen mit negativem szintigraphischen Befund, sowohl am Hals als auch am übrigen Körper, lag der Thyreoglobulinspiegel (initial) unter 5 ng/ml. Bei einem der 3 Patienten, die eine therapeutische Jod-131-Dosis erhalten hatten, wurde jedoch nach Radiojod ein Thyreoglobulinwert von 120 ng/ml gemessen (Abb. 2). Die Tomographie der Lunge deckte hier eine isolierte Metastase auf, die durch die Szintigraphie nicht erfaßt werden konnte (Abb. 3).

2. Gruppe „Schilddrüsenrest, Lokalrezidiv" (Abb. 1): Zeigt sich szintigraphisch am Hals in der Region des Schilddrüsenbettes Radioaktivitätsanreicherung, so kann es sich um zurückgebliebenes, normales Schilddrüsengewebe oder restliches Tumorgewebe, aber auch um ein Lokalrezidiv handeln. Der Thyreoglobulinspiegel lag bei 17 von insgesamt 25 dieser Fälle vor Radiojodtherapie unter 5 ng/ml, in

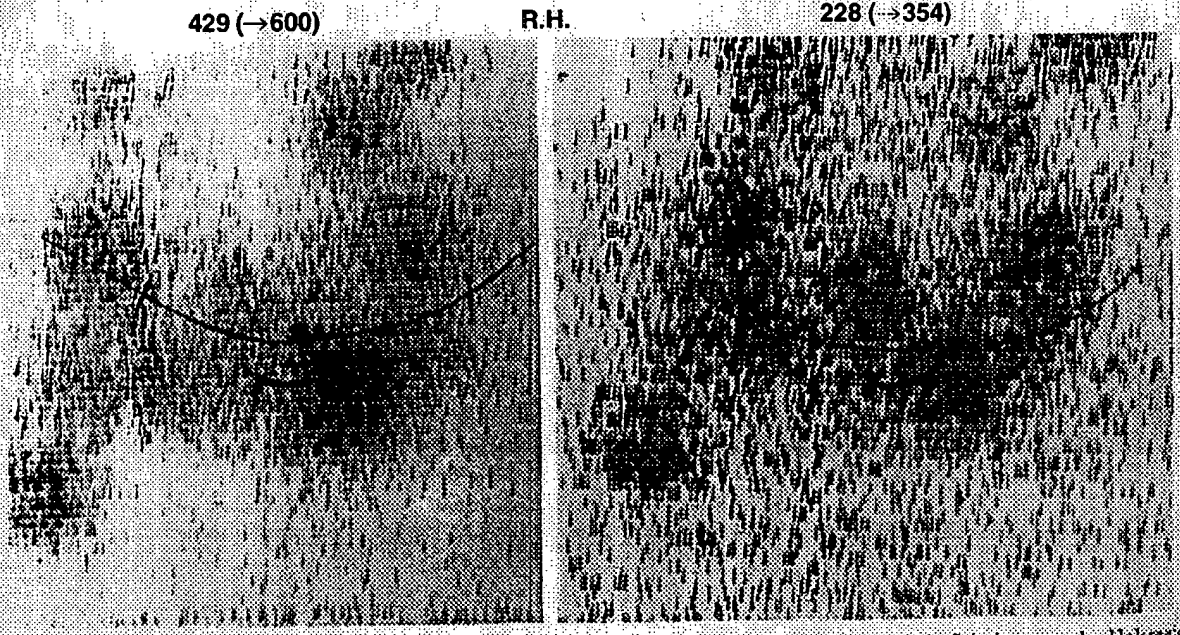

Abb. 6. Serumthyreoglobulinspiegel in ng/ml vor (in Klammer: nach) Radiojodtherapie und posttherapeutische Szintigramme der Halsregion zu zwei aufeinanderfolgenden Radiojodtherapie-Terminen. Während das szintigraphische Bild der regionalen Lymphknotenmetastasen (eingezeichnet Operationsnarbe und Jugulum) bei Beachtung der unterschiedlichen Technik die palpatorisch festgestellte Abnahme der Tumorgewebsmasse nicht erkennen ließ, fand sich ein Abfall des Thyreoglobulinspiegels von 429 auf 228 ng/ml (bzw. jeweils 10 Tage nach Radiojodtherapie von 600 auf 354 ng/ml)

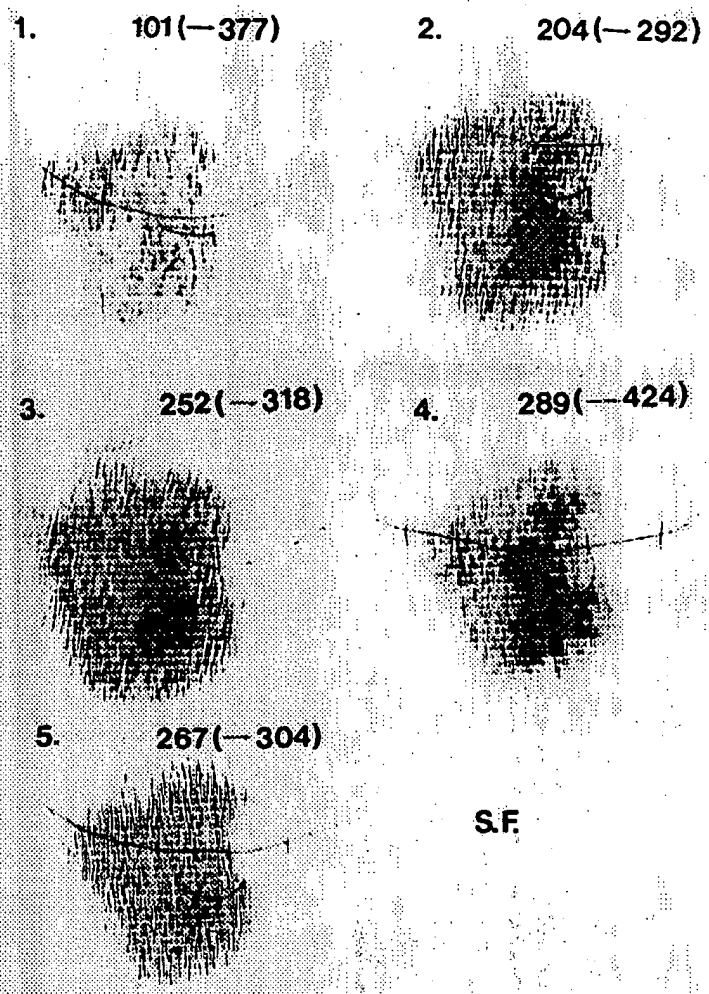

Abb. 7. Serumthyreoglobulinspiegel ng/ml vor (in Klammer: nach) Radiojodtherapie zu 5 Terminen bei einem Patienten mit Lokalrezidiv eines differenzierten Schilddrüsencarcinoms. Durch Serumthyreoglobulinmessung lassen sich die Änderungen in der Tumormasse besser quantitativ erfassen als durch die Szintigraphie

4 dieser Fälle war 10 Tage nach Radiojodgabe jedoch Thyreoglobulin nachzuweisen.

3. Gruppe „Metastasen" (Abb. 1): Unter den 8 Patienten mit szintigraphisch nachgewiesenen Metastasen lag Thyreoglobulin bei 4 Fällen sehr hoch, in weiteren 3 Fällen war es deutlich nachweisbar, einmal fand sich jedoch ein Meßwert unter 5 ng/ml: Bei dieser Patientin bestand eine szintigraphisch erfaßbare Metastasierung des papillären Carcinoms in die regionalen Lymphknoten (Abb. 4). Dennoch konnte hier Thyreoglobulin bei insgesamt 7 Messungen sowohl vor als auch 10 Tage nach Radiojodtherapie nicht gemessen werden. Endogene Thyreoglobulinantikörper, die als Ursache in Frage gekommen wären, wurden nicht gefunden.

Abbildung 5 zeigt den Verlauf der jeweils unmittelbar vor Radiojodtherapie gemessenen Thyreoglobulinwerte während der beiden letzten Jahre: Lag zu Beginn Thyreoglobulin, vor der Radiojodtherapie bestimmt, unter 5 ng/ml, so verblieb es – mit einer Ausnahme – auch später in diesem Bereich. (Zehn Tage nach der Therapie wurde hier allerdings in einem Teil der Fälle Thyreoglobulin nachweisbar). Umgekehrt wurden 3 Thyreoglobulin-positive Patienten später Thyreoglobulin-negativ. Drei der 4 Patienten mit Metastasen und sehr hohem Thyreoglobulinspiegel zeigten im Verlauf einen Abfall, zweimal kam es später zu einem – z.T. sehr starken – Wiederanstieg. Diese Schwankungen des Thyreoglobulinspiegels gingen in der Regel, aber durchaus nicht immer, mit dem Bild der Szintigramme parallel (Abb. 6). Auffallend war auch der Verlauf bei einem Patienten mit einem Lokalrezidiv, bei dem Thyreoglobulin trotz mehrfacher Radiojodtherapien ständig anstieg; erst nach der 4. Therapie wurde ein leichtes Absinken beobachtet. Die dazugehörigen Szintigramme entsprachen in etwa, jedoch nicht immer diesem Verlauf (Abb. 7). Speicherndes Gewebe außerhalb des Schilddrüsenbettes war nicht nachweisbar.

Bei insgesamt 3 der 40 Patienten wurden mit dem

Radioimmunoassay von CIS endogene Thyreoglobulinantikörper gefunden, die bei zwei der Patienten während des Beobachtungszeitraumes verschwanden. Ein Neuauftreten von Thyreoglobulinantikörpern konnte mit diesem Assay in keinem der Fälle nachgewiesen werden.

Diskussion

Finden sich nach Thyreoidektomie wegen differenzierten Schilddrüsencarcinoms hohe Serumspiegel an Thyreoglobulin, so zeigt dies eine Metastasierung an. Nach unseren Untersuchungen sowie denen anderer Autoren [1, 5] ist diese ab einem Thyreoglobulinwert von etwa 50–100 ng/ml anzunehmen. Allerdings kann trotz szintigraphisch nachweisbarer Metastasen der Thyreoglobulinspiegel wesentlich niedriger liegen, ja sogar unter der sicheren Nachweisgrenze wie bei einem unserer Fälle (Abb. 4) (vgl. auch [2, 5]). Zwischenzeitlich überblicken wir unter 10 Patienten mit szintigraphisch positiven, z.T. sogar sehr ausgedehnten Metastasen insgesamt 3 mit einem Thyreoglobulinspiegel unter 5 ng/ml.) Umgekehrt fanden wir unter 3 Patienten mit – wie sich im Nachhinein zeigte – negativem Szintigramm in einem Fall kurz nach der Radiojodtherapie Thyreoglobulin im Serum (vgl. auch [2, 3]). Hier ließ sich röntgentomographisch eine Lungenmetastase nachweisen (Abb. 2, 3). Offenbar kann es zu einer Dissoziation der verschiedenen Zell-Leistungen der pathologischen Abkömmlinge der Thyreozyten kommen: So könnte bei dem geschilderten Fall zwar noch das Jod-„Trapping" erhalten, aber der Jodeinbau sowie auch der Thyreoglobulin-Sekretionsmechanismus defekt gewesen sein; der celluläre Syntheseapparat für das Thyreoglobulin-Eiweißmolekül hingegen muß offenbar funktionsfähig geblieben sein, so daß das synthetisierte Thyreoglobulin nach Zellschädigung in den Blutkreislauf gelangen konnte. Da die TSH-Spiegel vor Radiojodtherapie bereits im (primär-) hypothyreoten Bereich gelegen waren, erscheint es wenig wahrscheinlich, daß der nach Radiojodtherapie nachweisbare Thyreoglobulinspiegel nur eine Folge einer eventuell jetzt verstärkten thyreotropen Stimulation gewesen ist.

Ähnliche Überlegungen wie oben können auch bezüglich der 4 erst nach Radiojodtherapie Thyreoglobulin-positiv gewordenen Fälle aus der Gruppe der Patienten mit Radiojodspeicherung im Schilddrüsenbett angestellt werden. Allerdings sollte man bedenken, daß auch knapp ein Viertel der Normalpersonen Thyreoglobulinwerte unter 5 ng/ml aufwies. Möglicherweise sind die derzeit verfügbaren Radioimmunoassays für Thyreoglobulin immer noch nicht genügend sensibel. Es ist auch zu diskutieren, daß die Thyreoglobulinmeßwerte zumindest bei einem Teil der Carcinom-Fälle durch endogene Thyreoglobulinantikörper, die mit dem verwendeten Radioimmunoassay von CIS nicht nachweisbar waren, verfälscht wurden.

Es erscheint uns wertvoll, auch kurz nach Radiojodtherapie, z.B. am Tage des posttherapeutischen Szintigramms, nochmals Thyreoglobulin im Serum zu messen, da sich dadurch ein Hinweis für die Existenz szintigraphisch nicht darstellbarer Metastasen ergeben kann. In derartigen Fällen erweist sich die Thyreoglobulinmessung dem Szintigramm somit als überlegen [3, 5].

Von unseren 40 Patienten mit differenzierten Schilddrüsencarcinomen waren insgesamt 20 Thyreoglobulin-negativ und 20 Thyreoglobulin-positiv. Während sich unter den 20 Thyreoglobulin-negativen Patienten nur einer mit Metastasen fand [12], ließ sich mit einer Ausnahme bei allen Thyreoglobulin-positiven Patienten szintigraphisch Tumor- bzw. Schilddrüsenrestgewebe nachweisen. Diese Daten zeigen die Wertigkeit und zugleich auch die Grenzen der Thyreoglobulinmessung für die Verlaufskontrolle beim Schilddrüsencarcinom: Nach totaler Entfernung des Schilddrüsengewebes ist die Bedeutung wesentlich größer als wenn Schilddrüsengewebe zurückbleibt.

Beim Vergleich der Thyreoglobulinspiegel mit den optisch beurteilten Veränderungen der Radiojodszintigramme ergab sich, daß die Thyreoglobulinmessung generell eine gute, quantitativ in Zahlen erfaßbare Abschätzung der Veränderungen der Tumorgewebsmasse gestattet. In einigen Fällen zeigten Szintigramme und Thyreoglobulinspiegel jedoch einen diskrepanten Verlauf, was durch die oben diskutierte, unterschiedliche Aktivität von Partialfunktionen der Thyreozytenabkömmlinge erklärlich erscheint.

Eine Interpretation der Meßwerte im Thyreoglobulin-Radioimmunoassay ist prinzipiell nur dann möglich, wenn keine endogenen Thyreoglobulinantikörper vorliegen [4, 7, 13]. Für die Testung auf Thyreoglobulinantikörper sollte wegen der höheren Sensibilität eine radioimmunologische Technik verwendet werden [9]. In den bisher publizierten Studien wurden die Patientenseren öfters nicht auf endogene Thyreoglobulinantikörper geprüft. Legt man unsere Meßergebnisse mit dem Radioimmunoassay von CIS für Thyreoglobulinantikörper zugrunde, so scheint dadurch kein übermäßig häufiger Störfaktor übersehen worden zu sein. Wir fanden mit dem CIS-Assay Thyreoglobulinantikörper nur bei 3 von 40 Patienten, bei 2 dieser Patienten verschwanden sie überdies im Verlauf der Beobachtung. Diese Resultate entsprechen auch denen anderer Autoren [2, 5, 12], allerdings wurde auch über eine höhere Inzidenz berichtet [7]. Aufgrund eigener Vergleichsuntersuchungen (Abb. 8) erscheint die von der Lieferfirma des verwendeten Thy-

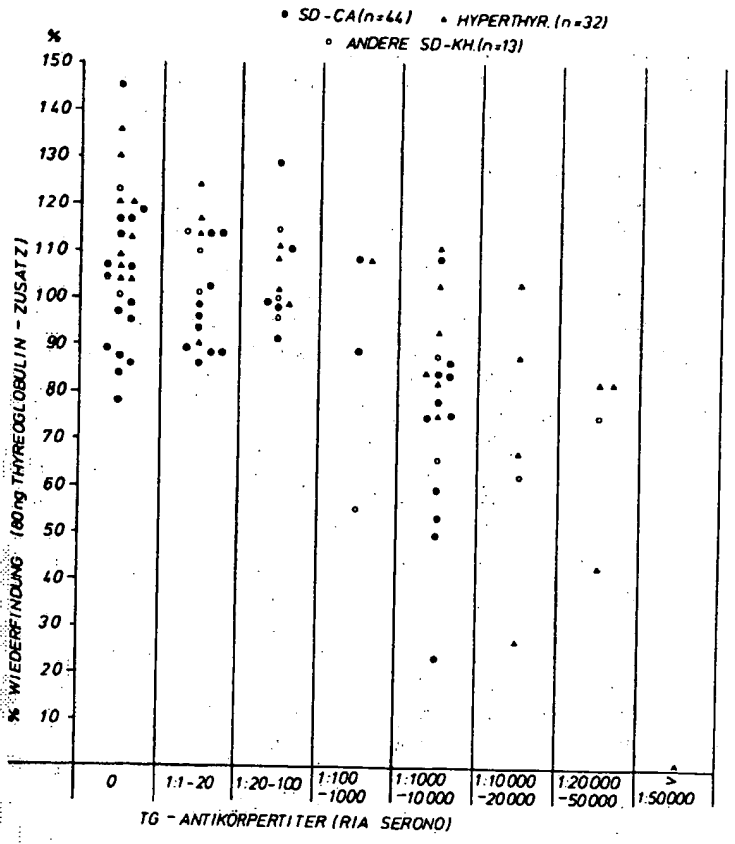

Abb. 8. Einfluß endogener Thyreoglobulin-Antikörper, bestimmmt mit dem Radioimmunoassay von Serono, auf die Wiederfindung von zugesetztem Thyreoglobulin in einem Thyreoglobulin-Doppelantikörper-Radioimmunoassay (Henning). Die Abbildung enthält die Ergebnisse von insgesamt 89 Seren von Patienten mit verschiedenen Schilddrüsenerkrankungen (von etlichen Patienten wurden die Werte mehrerer, zu verschiedenen Zeitpunkten abgenommener Serumproben eingezeichnet)

reoglobulin-Kits empfohlene Methode der Wiederfindung zugesetzten Thyreoglobulins (welche an sich ein Verfahren zur Überprüfung der Richtigkeit des Assays darstellt) nicht geeigneter, Thyreoglobulinantikörper-haltige Seren zu erfassen und somit von einer Interpretation auszuschließen. Der hochempfindliche Radioimmunoassay für Thyreoglobulinantikörper von Biodata (Serono), den wir in letzter Zeit zusätzlich verwenden (Abb. 8), zeigte zwar in einem deutlich größeren Teil der Seren Thyreoglobulinantikörper an, dann meist aber mit (sehr) niedrigem Titer. In diesen Seren war die Wiederfindung meist nicht gestört. Umgekehrt ergab sich öfters eine nicht 100%ige Wiederfindung, ohne daß mit den Assays von CIS oder Serono Thyreoglobulinantikörper nachweisbar waren (vgl. auch [7]). Mit dem Assay von Serono fanden wir unter mittlerweile 52 Patienten mit differenziertem Schilddrüsencarcinom 8 (= 15%) mit einem Thyreoglobulinantikörpertiter über 1:100.

Mit einiger Überraschung mußten wir feststellen, daß mit dem Radioimmunoassay von CIS – bei keinem der primär Antikörper-negativen Patienten im Beobachtungszeitraum das Neuauftreten von Thyreoglobulinantikörpern nachgewiesen werden konnte, trotz wiederholter Gewebsläsion mit Thyreoglobulinausschwemmung im Gefolge der Radiojodtherapien. Dies könnte bedeuten, daß bei einem intakten Immunsystem die Strahlenschädigung des Gewebes mit einer zeitlich begrenzten, vermehrten Ausschüttung des ohnedies physiologischen Sekretionsproduktes Thyreoglobulin nicht ausreicht, eine signifikante Antikörperbildung gegen Thyreoglobulin zu induzieren bzw. einen längeren Autoimmunprozeß in Gang zu setzen [10].

Tang Fui et al. [15] folgerten aus ihren Resultaten, daß ein Ganzkörperszintigramm unnötig sei, falls bei thyreoidektomierten Schilddrüsencarcinompatienten kein Thyreoglobulin im Serum nachweisbar sei (vergl. auch [3, 4a]). Dieser Meinung können wir uns aufgrund unserer Ergebnisse nicht anschließen. Da Schneider et al. [12] überdies fanden, daß der Thyreoglobulinspiegel 14 Tage nach Absetzen, nicht jedoch unter einer Trijodthyroninmedikation ein brauchbarer Indikator für radiojodspeicherndes Gewebe sei, stellen alleinige Thyreoglobulinmessungen unter fortlaufender Schilddrüsenhormontherapie nach unserer Meinung keine generell bzw. stets ausreichende Nachsorgemaßnahme dar (vgl. auch [2, 5]).

Insgesamt kann Thyreoglobulin bei Patienten mit differenziertem Schilddrüsencarcinom nach Thyreoidektomie als Tumormarker betrachtet werden. Die Thyreoglobulinbestimmung vermag jedoch die Ganz-

körperszintigraphie nach Radiojodgabe sicherlich nicht zu ersetzen [2], wenn sie sich auch in manchen Fällen der Szintigraphie als überlegen erweist [3]. Auch gelingt die Abschätzung der Änderungen der Tumorgewebsmasse mit der Thyreoglobulinmessung manchmal besser als mit der Szintigraphie. Es wird daher die Kombination der Szintigraphie nach Radiojodgabe mit der radioimmunologischen Thyreoglobulinmessung für die Verlaufskontrolle von Patienten nach Thyreoidektomie wegen differenzierten Schilddrüsencarcinoms empfohlen.

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Literatur

1. Botsch H, Schulz E, Lochner B (1979) Serum-Thyreoglobulinbestimmung zur Verlaufskontrolle bei Schilddrüsencarcinom-Patienten. Dtsch Med Wochenschr 104:1072–1074
2. Bridgman MC, Cooper RA, Luttrell BM, Reeve TS, Stiel JN, Wilmshurst EG, Hales IB (1980) Evaluation of serum thyroglobulin levels in the follow-up of thyroid cancer following ablative therapy. In: Stockigt JR, Nagataki S (eds) Thyroid research VIII. Proc 8th Int Thyroid Congr, Sydney 1980. Austral Acad Science, Canberra, pp 483–485
3. Charles MA, Dodson LE Jr, Waldeck N, Hofeldt F, Ghaed V, Telepak R, Owenbey J, Burstein P (1980) Comparison of the serum thyroglobulin radioimmunoassay to total body iodine scans in patients with treated well-differentiated thyroid carcinoma. In: Stockigt JR, Nagataki S (eds) Thyroid Research VIII Proc 8th Int Thyroid Congr, Sydney 1980. Austral Acad Science, Canberra 1980, pp 486–489
4. Gärtner R, Horn K, Pickhardt RC (1980) Improvement of the diagnostic validity of the thyroglobulin radioimmunoassay. Acta Endocrinol (Copenh) [Suppl 234] 94:30–31 (Abstract)

4a. Hagemann J, Schneider C (1978) Control of treatment of differentiated thyroid carcinoma by measurement of thyroglobulin in serum. In: Radioimmunoassay and related procedures in medicine 1977, Vol II. Int Atomic Energy Agency, Vienna (IAEA-SM-220/23), pp 363–368

5. Hüfner M, Pollmann H, Grussendorf M, Schenk P (1980) Die Bedeutung der Thyreoglobulinbestimmung im Serum bei der Nachsorge von Patienten mit differenziertem Schilddrüsencarcinom. Schweiz Med Wochenschr 110:159–162
6. Lo Gerfo P, Stillman T, Colacchio D, Feind C (1977) Serum thyroglobulin and recurrent thyroid cancer. Lancet I:881–882
7. Reiners C, Börner W, Baum K, Stock KD, Moll E (1980) Spezifische und unspezifische Tumormarker zur Verlaufskontrolle des Schilddrüsenmalignoms. Vortrag Ges f Nuklearmedizin, Nürnberg, Sept 1980
8. Roitt IM, Torrigiani G (1967) Identification and estimation of undegraded thyroglobulin in human serum. Endocrinology 81:421–429
9. Schatz H (1978) Methodik und Wertigkeit der Bestimmung von Schilddrüsenantikörpern. Der Nuklearmediziner 1:40–51
10. Schatz H (1980) Die chronische lymphozytäre Thyreoiditis (Hashimoto). Intern Welt 3:348–358

10a. Schatz H, Schröder O, Grebe S (1981) Thyroglobulin levels in sera of patients with differentiated thyroid carcinoma. Longitudinal and cross sectional studies. Acta Endocrinol (Copenh) [Suppl 240] 96:14 (Abstract)

11. Schneider AB, Favus MJ, Stachura ME, Arnold JE, Yun Ryo U, Pinsky S, Colman M, Arnold MJ, Frohman LA (1977) Plasma thyroglobulin in detecting thyroid carcinoma after childhood head and neck irradiation. Ann Intern Med 88:29–34
12. Schneider AB, Goldman JM, Line BR, Robbins J (1980) Serum thyroglobulin and ^{131}I testing in the management of patients with thyroid cancer. Endocrinology [Suppl] 107:T5 (Abstract)
13. Schneider AB, Pervos R (1978) Radioimmunoassay of human thyroglobulin: Effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab 47:126–137
14. Shlossberg AH, Jacobson JC, Ibbertson HK (1979) Serum thyroglobulin in the diagnosis and management of thyroid carcinoma. Clin Endocrinol 10:17–27
15. Tang Fui SCN, Hoffenberg R, Maisey MN, Black EG (1979) Serum thyroglobulin concentrations and whole-body radioiodine scan in follow – up of differentiated thyroid cancer after thyroid ablation. Br Med J II:298–300
16. Van Herle AJ, Uller RP (1975) Elevated serum thyroglobulin. A marker of metastases in differentiated thyroid carcinomas. J Clin Invest 56:272–277
17. Van Herle AJ, Uller RP, Matthews NL, Brown J (1973) Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest 52:1320–1327
18. Van Herle AJ, Vassart G, Dumont JE (1979) Control of thyroglobulin synthesis and secretion. (Part I and II). N Engl J Med 301:239–249, 307–314

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